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Interferon-α receptor 1 mRNA expression in peripheral blood mononuclear cells is associated with response to interferon-α therapy of patients with chronic hepatitis C

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Abstract

Interferon (IFN)-α receptor mRNA expression in liver of patients with chronic hepatitis C has been shown to be a response to IFN-α therapy. The objective of the present study was to determine whether the expression of mRNA for subunit 1 of the IFN-α receptor (IFNAR1) in peripheral blood mononuclear cells (PBMC) is associated with the response to IFN-α in patients with chronic hepatitis C. Thirty patients with positive anti-HCV and HCV-RNA, and abnormal levels of alanine aminotransferase in serum were selected and treated with IFN-α2b for one year. Those with HBV or HIV infection, or using alcohol were not included. Thirteen discontinued the treatment and were not evaluated. The IFN-α response was monitored on the basis of alanine aminotransferase level and positivity for HCV-RNA in serum. IFNAR1-mRNA expression in PBMC was measured by reverse transcription-polymerase chain reaction before and during the first three months of therapy. The results are reported as IFNAR1-mRNA/ß-actin-mRNA ratio (mean ± SD). Before treatment, responder patients had significantly higher IFNAR1-mRNA expression in PBMC (0.67 ± 0.15; N = 5; $P < 0.05$) compared to non-responders (0.35 ± 0.17; N = 12) and controls (0.30 ± 0.16; N = 9). Moreover, IFNAR1-mRNA levels were significantly reduced after 3 months of treatment in responders, whereas there were no differences in IFNAR1 expression in non-responders during IFN-α therapy. Basal IFNAR1-mRNA expression was not correlated with the serum level of alanine and aspartate aminotransferases or the presence of cirrhosis. The present results suggest that IFNAR1-mRNA expression in PBMC is associated with IFN-α response to hepatitis C and may be useful for monitoring therapy in patients with chronic hepatitis C.

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- Peripheral blood mononuclear cells
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Hepatitis C virus (HCV) causes chronic liver infection in approximately 2% of the world population (1). Until recently, chronic hepatitis C was treated with interferon (IFN)-α monotherapy, but the combination therapy with ribavirin increases the sustained-response rate and therefore is currently the recommended treatment for HCV infection (2). The response to IFN-α is influenced both by viral virulence factors and by the efficacy of the host immune response to HCV (3). IFN-α activity is mediated by its high affinity binding to specific cellular receptors (IFNAR) and subsequent triggering of the signaling pathway that induces the antiviral state in the cell (4). Altered production of IFN-α and IFN-induced antiviral proteins, presence of anti-IFN-α antibodies in plasma and other host responses related to the IFN-α system have been implicated in the resistance to antiviral therapy (5,6). It has been shown that soluble human IFNAR subunits 1 (IFNAR1) and 2 (IFNAR2) inhibit IFN-α antiviral activity *in vitro* (7,8) and suppress the effectiveness of IFN-α therapy in patients with chronic hepatitis C (9).

IFNAR-mRNA expression in liver of patients with chronic hepatitis C has been shown to be related to the response to IFN-α therapy. Absent or low intrahepatic IFNAR-mRNA levels are related to a poor response to IFN-α, to increased histological hepatitis activity and to the severity of liver fibrosis in patients with chronic hepatitis C (10-12). In addition, IFNAR-mRNA expression in liver is correlated negatively with HCV-RNA viral load and the presence of HCV genotype 1b in HCV-infected patients (13-15).

IFNAR1-mRNA has been detected in peripheral blood mononuclear cells (PBMC) from patients with chronic hepatitis C; however, no association with response to IFN-α was demonstrable (10). Using a semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assay, we have analyzed IFNAR1-mRNA expression in PBMC from patients chronically infected with HCV and demonstrated its association with the IFN-α response.

Thirty patients with chronic hepatitis C according to diagnostic criteria (abnormal level of serum alanine aminotransferase, ALT, and positive anti-HCV and HCV-RNA in serum) were selected from 1998 to 2000 at the Gastroenterology Service of the São Paulo Hospital, UNIFESP (São Paulo, SP, Brazil), and those with hepatitis B virus or human immunodeficiency virus infection, or using alcohol were not included. Individuals with negative tests for HCV infection and other liver diseases were selected for the control group (N = 9). Recombinant IFN-α2b (Biosintética S.A., São Paulo, SP, Brazil) was given to all HCV-infected patients for a period of one year (3 MU, intramuscular, three times a week). The protocol was approved by the local Ethics Committee and all individuals gave written informed consent before entering the study. IFN-α therapy was monitored at three-month intervals by the RNA-HCV test and on the basis of serum levels of ALT, a marker of liver injury. Patients with abnormal ALT and positive RNA-HCV by the third month (N = 12) were considered non-responders. Those with normal ALT and negative RNA-HCV after 6 months of treatment were classified as responders (N = 5). Thirteen patients discontinued treatment and could not be evaluated. The measurement of serum levels of liver enzymes and HCV-RNA tests was performed as described (16).

RNA was extracted from PBMC (10^6-10^7 cells) using the TRIzol® Reagent (Life Technologies, Gaithersburg, MD, USA). cDNA was produced from 3 µg of total RNA by RT using SuperScript™ RNase H^- RT (Life Technologies). The PCR preparations contained 3 µl cDNA, 0.1 µM specific primers (12), 200 µM dNTPs, and 2.5 U *Taq* DNA polymerase (Amersham Pharmacia Biotech, Uppsala, Sweden). Amplification was carried out over 42 (IFNAR1) and 25 (ß-actin) cycles at 95ºC for 1 min, 52ºC for 2

min, and 72ºC for 2 min, followed by 72ºC for 10 min for final extension. PCR products were separated by 1.0% agarose gel electrophoresis and analyzed by absorbance using a GS-700 Image Analyzer (Bio-Rad, Hercules, CA, USA). IFNAR1-mRNA level in PBMC was assessed as the IFNAR1-mRNA/ß-actin-mRNA ratio. The result reported for each RNA sample is the mean of at least three RT-PCR assays.

According to the data shown in Table 1, age and basal levels of liver enzymes were higher ($P < 0.05$) in HCV-infected patients than in controls. Serum ALT and aspartate aminotransferase (AST) are usually increased in chronic hepatic injury, while high levels of alkaline phosphatase and gamma-glutamyltransferase are associated with cholestasis (2). Age, gender, cirrhosis and basal levels of serum liver enzymes were not associated with the response to IFN-α therapy (Table 1; $P > 0.05$). In contrast, serum ALT and AST levels were significantly reduced during treatment in responders ($P < 0.05$; data not shown), demonstrating the usefulness of ALT for monitoring chronic hepatitis C treatment (2).

Responder patients had higher basal levels of IFNAR1-mRNA in PBMC than non-responders and controls (Table 1; $P < 0.05$). The differences in IFNAR1-mRNA levels in PBMC between responders and non-responders before IFN-α therapy were similar to those observed in liver biopsies from HCV-infected patients (14,17). It is likely that IFNAR1-mRNA expression in PBMC reflects the IFNAR1-mRNA status of the liver, as recently demonstrated for IFNAR2 (18).

It has been suggested that lower IFNAR1-mRNA levels in non-responders before treatment may result from a primary disorder of endogenous IFN-α production in liver and PBMC from HCV patients (5). However, an experimental study demonstrated that disruption of the IFNAR1 gene leads to high susceptibility to several viruses (19). This indicates that the deficient production of IFNAR1 transcripts may be the primary defect that leads to the lack of a response to IFN-α in HCV-infected patients. In fact, a significant reduction of IFNAR1-mRNA and -protein levels in liver has been associated with a high histological hepatitis activity, advanced liver fibrosis, poor response to IFN-α, and high viral load (12,17). In addition, HCV patients co-infected with serologically silent hepatitis B virus have lower IFNAR-mRNA levels in the liver and an unfavorable response to IFN-α (20) than non-co-infected patients. This effect was probably caused by down-regulation of IFNAR gene expression in the liver due to the presence of HBV co-infection.

Basal IFNAR1-mRNA expression in PBMC did not correlate with the presence of cirrhosis or liver enzyme levels for either responders or non-responders ($P > 0.05$; data not shown). Other studies have also reported no association between IFNAR1-mRNA expression in liver and the presence of cirrhosis or the degree of histological fibrosis in

Table 1. Clinical and laboratory data of chronic hepatitis C patients before interferon-α therapy.

	Responders	Non-responders	Controls
Number of patients	5	12	9
Age (years)[a]	54 ± 15	54 ± 10	27 ± 8*+
Sex (male/female)[b]	2/3	7/5	2/7
Serum enzymes (ULN)			
ALT[a]	1.7 ± 0.8	2.8 ± 1.7	0.3 ± 0.1*+
AST[a]	2.0 ± 0.7	2.8 ± 1.3	0.4 ± 0.1*+
ALP[a]	1.1 ± 0.5	0.9 ± 0.3	0.5 ± 0.2*+
GGT[a]	2.2 ± 1.0	2.5 ± 1.0	0.7 ± 0.2*+
IFNAR1-mRNA in PBMC[a]	0.67 ± 0.15	0.35 ± 0.17*	0.30 ± 0.16*
Liver biopsy			
Cirrhosis (Yes/No)[b]	2/3	8/4	-

Data are reported as means ± SD for the number of individuals indicated in the first entry to the table. ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; GGT = gamma-glutamyltransferase; IFNAR1-mRNA = interferon-α-receptor 1-mRNA; PBMC = peripheral blood mononuclear cells; ULN = upper limit of normal range. ALT-ULN = 50 U/l; AST-ULN = 46 U/l; ALP-ULN = 250 U/l; GGT-ULN = 28 U/l (men) and 18 U/l (women). *$P < 0.05$ *vs* responder patients; +$P < 0.05$ *vs* non-responder patients ([a]Kruskal-Wallis test followed by the Dunn test; [b]chi-square test).

the liver (13,14,17). Moreover, IFNAR-mRNA levels in liver were not correlated with serum ALT or 2',5'-oligoadenylate synthetase levels, viral load or HCV (11,17).

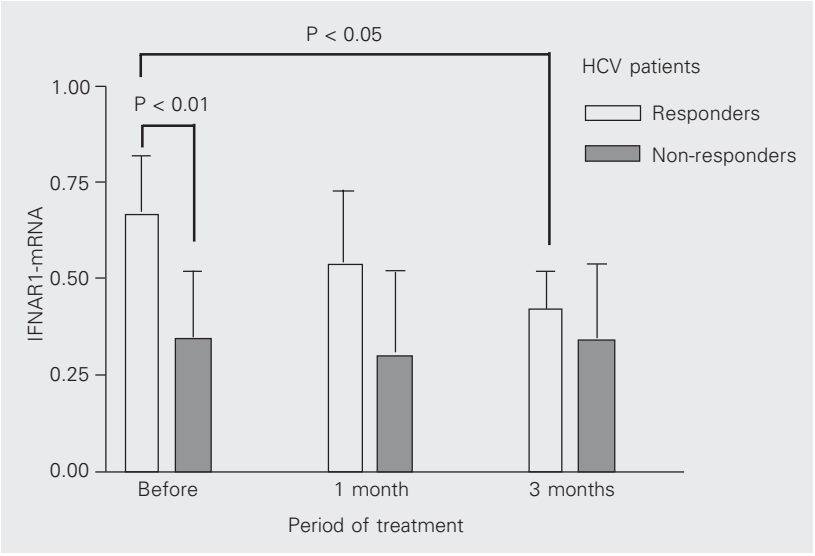

Figure 1. Interferon-α-receptor 1-mRNA (IFNAR1-mRNA) levels in peripheral blood mononuclear cells from 5 responder (open columns) and 12 non-responder (filled columns) patients with chronic hepatitis C before and during a three-month period of interferon-α therapy. Data are reported as means ± SD and compared by the Student *t*-test. Comparisons are indicated in the Figure. Responder = normal ALT and negative RNA-HCV in serum, and non-responder = abnormal ALT and positive RNA-HCV, after therapy.

Responder patients presented a significant reduction of IFNAR1-mRNA levels in PBMC at the third month of IFN-α therapy that was not seen in non-responders (Figure 1). After this period, the level of IFNAR1-mRNA expression in responders remained constant up to the end of one year of treatment. Therefore, exposure to high levels of exogenous IFN-α leads to the down-regulation of IFNAR1 expression in responders. In contrast, this mechanism fails in non-responder patients, probably due to a primary defect in production of IFNAR1 transcripts. These results suggest that IFNAR1-mRNA expression in PBMC may be useful for monitoring IFN-α therapy.

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