Induction of IL-1Ra in Resistant and Responsive Hepatitis C Patients Following Treatment with IFN-con1

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ABSTRACT

Hepatitis C virus (HCV) infection is resistant to interferon-α (IFN-α) in some patients. The mechanism of this resistance is unknown. Interleukin-1 receptor antagonist (IL-1Ra) is induced by IFN-α and is a good indicator of IFN activity. In the current study, we compared IL-1Ra levels in rapid virologic responders and flat responders who showed resistance to IFN. Three groups of patients were examined, including those who received a single dose of consensus IFN (IFN-con1), patients who received daily IFN-con1 for 1 week, and patients who received IFN-con1 daily for 24 weeks. Serum IL-1Ra, IL-6, and HCV RNA were measured serially in all groups. Serum IL-1Ra levels increased rapidly in all patients with hepatitis C after IFN-α administration, irrespective of their virologic response. IL-1Ra levels remained elevated at 1 week but were similar to baseline by week 2 of treatment in patients receiving continuous therapy. IL-6 levels also increased acutely but rose more slowly than IL-1Ra levels. The increase in IL-1Ra and IL-6 observed in both flat and rapid virologic responders indicates that IFN receptors are functioning in patients with IFN-resistant hepatitis C and that the lack of response is related to other virologic or immunologic factors.

INTRODUCTION

Treatment of hepatitis C virus (HCV)-infected patients with interferon-α (IFN-α) for 48 weeks leads to sustained response rates of approximately 20%.1–3 Factors associated with a virologic response include HCV genotype, pretreatment HCV RNA level, degree of fibrosis on liver biopsy, and gender.4 Biologic mechanisms leading to sustained response in some patients and lack of response in other patients are not understood. Genetic factors, including the availability of IFN receptors, differential induction of IFN-induced genes, and cytokine production, may all be involved. Recent viral kinetic studies indicate that IFN-α causes a rapid dose-dependent first-phase decline in HCV RNA level within 24–48 h in a subgroup of patients (rapid responders).5,6 This fast decline appears to be due to a direct effect of IFN-α on viral replication. However, HCV infection shows resistance to IFN-α in other patients who have little decline in viral titer during this initial period (flat responders). A slow second-phase decline in viral level follows the initial response in some patients and has been attributed to the clearance of infected hepatocytes. The mechanisms associated with the second-phase response are not known but may be related to either a cytokine or immunologic response.

We have analyzed the cytokine response in patients treated with consensus IFN (IFN-con1) immediately after initiation of treatment and at later times during therapy. IFN did not cause acute changes in serum interleukin-2 (IL-2), IL-4, tumor necrosis factor-α (TNF-α), or IFN-γ levels.7 However, serum IL-6 levels increased within 6–8 h in all treated patients. This increase was transient, and IL-6 levels returned to baseline by 24 h even with continuous IFN-α therapy.7

In this paper, we report similar results with IL-1 receptor antagonist (IL-1Ra). IL-1Ra levels became elevated in the serum of all HCV-infected patients within 4–6 h of initiation of INF-α treatment irrespective of their first-phase virologic response. In a group of patients receiving daily injections of IFN-con1, IL-1Ra levels remained elevated at 1 week of treatment and

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were not significantly different from baseline by week 2. The observation that responses associated with IFN-α induction occurred even in the absence of an appreciable viral reduction in flat responders indicates that resistance to IFN-α was due to mechanisms other than a lack of IFN receptors in these patients.

MATERIALS AND METHODS

In Institutional Review Boards at RUSH-Presbyterian-St. Luke’s Medical Center, the Copernicus Group Independent Review Board, and Indiana University approved the research protocol. All subjects provided written informed consent before study entry.

Subjects

**Group 1.** Eighteen patients with chronic hepatitis C, aged 54 ± 11 years, who were previous virologic nonresponder to IFN therapy were included. All subjects had detectable hepatitis C viremia, genotype 1 infection, and histologic features of hepatitis C and lacked evidence of other causes of liver disease. Nine subjects received a single 15-µg dose of IFN-con1, and 9 received a single 30-µg dose. Serum samples were collected at time 0 (baseline) and 1, 2, 4, 6, 8, 24, 48, 72, and 96 h postinjection.

**Group 2.** Seven treatment naive patients with hepatitis C viremia, histologic evidence of chronic hepatitis C, and no features of other causes of liver disease were evaluated. Their mean age was 48 ± 7 years. All had HCV genotype 1 infection. Each patient received 15 µg of IFN-con1 daily. Blood samples were collected at 0, 6, 15, 21, 24, 48, and 96 h and at 1 week of treatment, and serum was stored at −70°C until time of testing.

**Group 3.** Twenty treatment naive subjects with hepatitis C viremia and genotype 1 infection were included. Their mean age was 47 ± 6 years. All subjects were African American and received either 9 or 15 µg IFN-con1 at daily intervals. Blood samples were taken at baseline and at weeks 1, 2, 12, and 24, and serum was stored at −70°C until time of testing.

**Control subjects.** Fifty healthy student and faculty volunteers who had blood drawn at the Indiana University Health Center served as controls. Blood samples were treated identically to those of subjects in the clinical trials.

Cytokine assays

ELISA kits were purchased from R & D Systems (Minneapolis, MN). The assays were performed with standard cytokines supplied by the manufacturer. The lower limit of detection for the IL-1Ra ELISA was 25 pg/ml. IL-6 measurements were performed by high-sensitivity ELISA with a detection limit of 0.7 pg/ml, as described previously.(7)

Virologic testing

HCV RNA levels were measured by a quantitative polymerase chain reaction (PCR) assay with a range between 10^2 and 5 × 10^6 copies per milliliter (National Genetics Institute, Los Angeles, CA). Patients who had >1 log_{10} reduction in HCV RNA level during the first 48 h of treatment were classified as rapid responders. Flat responders had <1 log_{10} decline during the first 48 h after receiving IFN-con1.

**RESULTS**

**IL-1Ra levels**

IL-1Ra levels were similar at baseline in healthy controls (median 344 pg/ml, interquartile range 294–437 pg/ml) and in patients with hepatitis C (median 445 pg/ml, interquartile range 324–868 pg/ml). Serum IL-1Ra increased within 4 h of IFN-con1 administration in group 1 and reached levels as high as 20–30 times that of baseline samples in some cases by 6–8 h (Fig. 1). The levels returned to baseline by 48 h in the majority of patients. There was a trend toward greater IL-1Ra levels at 24 h in group 1 participants who received 30 µg IFN-con1 compared with those who received the 15-µg dose (p = 0.06). In group 2 patients (n = 7) receiving continuous daily treatment with IFN-con1, IL-1Ra levels remained slightly elevated at week 1 (Fig. 2A).

In combining data from groups 2 and 3, IL-1Ra levels increased significantly from baseline (median 210 pg/ml, interquartile range 54–622 pg/ml) to week 1 (median 383 pg/ml, interquartile range 238–908 pg/ml) of continuous IFN treatment (p = 0.001). However, in group 3, where samples were taken at weekly intervals, there was no significant difference in IL-1Ra levels between baseline (median 210 pg/ml, interquartile range 54–447 pg/ml) and week 2 (median 244 pg/ml interquartile range 202–577 pg/ml) or week 12 (median 348 pg/ml, interquartile range 286–368 pg/ml) (Fig. 2B).

IL-1Ra levels increased in all subjects after IFN-con1 administration, even in flat responders who had little reduction in HCV RNA levels during the first 48 h after dosing. Table 1 shows changes in HCV RNA titers and IL-1Ra and IL-6 levels in both flat and rapid responders during the first 24 h of treatment. Table 2 presents similar data from group 3 patients, where samples were taken on a weekly basis. IL-6 levels were at baseline after 1 week and are not shown in Table 2.

Group 3 patients who were treated with 15 µg IFN-con1 daily tolerated this treatment well. No patient dropped out of the study because of IFN-con1 side effects. The most common side effects were fatigue, malaise, and irritability. Alanine aminotransferase (ALT) levels never completely normalized in the majority of the patients. In 9 patients, ALT levels normalized at various times between 1 week and 16 weeks. However, this could not be directly correlated with viral load. In many patients, ALT levels increased at later times.

**DISCUSSION**

There is extensive evidence that HCV-specific cytotoxic CD8 cells are present and active in persons with hepatitis C infection.(8–10) An ineffective cellular immune response causes ongoing liver injury in patients with chronic HCV. IFN acts in part by enhancing the immune response directed against viral
FIG. 1. Serum IL-1Ra levels after a single 15-μg (A) or 30-μg (B) injection of IFN-con1.
antigens, which may include the production of specific cytokines, regulation of cytokine receptors, and activation of natural killer (NK) cells and macrophages. Cytokine responses and resulting cytotoxic T cell activation may lead to viral clearance in some cases.

We have initiated a systematic analysis of cytokines induced during treatment with IFN-con1. Previous evaluation of serum samples from three groups of patients showed no significant induction of IL-2, IL-4, IFN-γ, or TNF-α. We have also examined serum levels of IL-16 and IL-18 in patients treated with IFN-con1 and did not find any increase (data not shown). We previously reported elevated levels of the proinflammatory cytokine IL-6 following treatment and in this paper, we report that the level of IL-1Ra increases with INF-con1 administration.

Naveau et al. reported that IL-1Ra levels increase with IFN-2α therapy. In contrast to our findings, Naveau et al. observed peak IL-1Ra levels at 11 days of treatment, and IL-1Ra remained elevated throughout 4 months of therapy. However, they first measured IL-1Ra 11 days after initiation of therapy (as opposed to our hourly samples in group 1 and weekly samples in group 3), and subsequent IL-1Ra levels were only 2–3-fold over baseline. In the present study, IL-1Ra values increased as much as 10–20-fold in some patients at week 1. Shiffman et

FIG. 2. Serum IL-1Ra measurements at 1 week (A) and 24 weeks (B) of continuous IFN-con1 therapy.
recently reported a difference in cytokine secretion from cultured phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC) at 24 h treated with either IFN-2b or IFN-con1. Whereas IFN-2b increased the secretion of IL-2, TNF, and IFN-γ by 4.5-fold, 4.1-fold, and 8.3-fold, the increase after treatment with IFN-con1 was only 1-fold, 1.9-fold, and 1.9-fold, respectively, above control levels. We previously showed that IFN-con1 induces IL-1Ra in whole blood in vitro. IFN-con1 appeared to be a more efficient inducer of IL-1Ra than IFN-α2b in vitro. Previous studies that showed an increase in IL-1Ra levels after treatment with IFN-α did not evaluate the relationship between IL-1Ra induction and changes in HCV RNA level. The data presented here provide new evidence that IFN-con1 induces IL-1Ra irrespective of the virologic response. Taken together, the findings of this study and our previous report show that IL-1Ra AND IFN-con1.

<table>
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<th>Time (h)</th>
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<th>Patient 19</th>
<th>Patient 20</th>
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<tr>
<td></td>
<td>RNA titer (copies/ml)</td>
<td>IL-1Ra (pg/ml)</td>
<td>IL-6 (pg/ml)</td>
<td>RNA titer (copies/ml)</td>
<td>IL-1Ra (pg/ml)</td>
<td>IL-6 (pg/ml)</td>
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<td>1,900,000</td>
<td>2,000,000</td>
<td>2,600,000</td>
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<td>IL-1Ra (pg/ml)</td>
<td>622</td>
<td>862</td>
<td>828</td>
<td>124</td>
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<td>669</td>
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Third patterns of viral response have been noted with IFN-α treatment. One response is an initial rapid response with a 1–2 log₁₀ reduction in HCV RNA titer within the first 24–48 h after treatment is initiated. HCV levels then either continue to decline and become undetectable in end-treatment responders or fail to decrease further or increase in partial responders. A third group of patients shows no appreciable reduction in HCV RNA level (flat responders) and is considered resistant to IFN therapy. This absence of response to IFN could be due to a lack of IFN receptors, to the presence of a resistant HCV strain, or to the ability of the virus to knock out IFN-induced genes, such as RNA-activated protein kinase (PKR). The induction of IL-1Ra is a good indicator of INF-α activity. We found that both IL-1Ra and IL-6 were induced rapidly in flat responders as well as in rapid responders to IFN-con1. These findings indicate that IFN receptors are functioning in patients who show resistance to IFN-α and that one or both of the other two proposed mechanisms for resistance must be involved.

IL-1Ra is induced by a number of cytokines, including IFN-α, IL-6, and granulocyte colony-stimulating factor (G-CSF). Previous studies that showed an increase in IL-1Ra levels after treatment with IFN-α did not evaluate the relationship between IL-1Ra induction and changes in HCV RNA level. The data presented here provide new evidence that IFN-con1 induces IL-1Ra irrespective of the virologic response. Taken together, the findings of this study and our previous report show that IL-1Ra AND IFN-con1.
a lack of association between systemic cytokine responses and the antiviral effect of IFN therapy.

IL-1Ra is produced by differential splicing from a gene coding for both an intracellular and a soluble form of the protein. The 17-kDa soluble form (sIL-1Ra) is produced by hepatocytes and is regulated by proinflammatory cytokines, such as IL-6 and IL-1. IL-1Ra is regulated in hepatocytes by acute-phase proteins and is produced by a number of cells of the monocyte lineage. We found that IL-6 levels also increased with IFN-con1 administration in all patients irrespective of the viral response. It is possible that the elevated levels of IL-1Ra reported in this study are related to induction of IL-6, although the increase in IL-6 lagged behind the rise in IL-1Ra levels.

There is limited information about the pattern of cytokine production in patients with hepatitis C. Even less is known about the effect of IFN-α on the induction or suppression of cytokines and whether this is related to virologic response. Studies that have evaluated serum cytokine levels after IFN-α administration have generally measured specific cytokines before treatment and 6 months later, at the end of the treatment period. Changes in serum cytokine levels during the course of therapy have not been well characterized. No major differences in cytokine responses have been found to date between responders and nonresponders.

In conclusion, our data provide evidence that IFN receptors are functioning in patients with HCV infection that is resistant to IFN-con1 therapy, indicating that other virologic or immunologic factors must be involved in the lack of response.

REFERENCES


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