Effect of Alpha Interferon (IFN- α) on 2'-5' Oligoadenylate Synthetase Activity in Peripheral Blood Mononuclear Cells of Patients with Chronic Hepatitis C: Relationship to the Antiviral Effect of IFN- α

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Alpha interferon (IFN- α) is, to date, the only treatment with proven efficacy in patients with chronic hepatitis C. However, less than 15% of the patients have a sustained response to IFN- α . Interferon acts through the induction of various cellular enzymes. Among them, the 2'-5' oligoadenylate synthetase (2-5OAS) is (at least in part) responsible for a direct antiviral effect of IFN- α . The aim of this study was to determine whether basal and IFN- α -induced in vivo and in vitro 2-5OAS activities measured in peripheral blood mononuclear cells predict biochemical and virological responses to IFN- α in patients with chronic hepatitis C. 2-5OAS activity in peripheral blood mononuclear cells and the antiviral effect of IFN- α were studied in 36 patients with chronic hepatitis C (27 men and 9 women; mean age, 44.7 years). Basal in vivo 2-5OAS activity (mean $\pm$ standard error of the mean) was 4.41 ± 0.69 nmol/10⁶ cells. It was significantly induced at month 3 of IFN- α therapy (18.07 ± 2.74 nmol/10⁶ cells; $P = 0.0001$). No significant differences were found in basal in vivo 2-5OAS activities, in IFN- α -induced/basal in vitro 2-5OAS activity ratios, in IFN- α -induced in vivo 2-5OAS activities, and in IFN- α -induced/basal in vivo 2-5OAS activity ratios between the patients with and without a biochemical response (normal alanine aminotransferase activity in serum) or a virological response (normal alanine aminotransferase activity in serum and negative hepatitis C virus RNA detection) at any step of the study. At month 3 of therapy, p69, which is considered to be the active isoform of 2-5OAS, was induced, as demonstrated by Western blot (immunoblot) analysis in 50% of the patients, and induction of the p100 isoform was observed in 70% of the patients. No significant relationship with the response to IFN- α therapy was observed. Our results suggest that a deficiency of the IFN- α -dependent 2-5OAS system, which could be genetically determined, is unlikely to be responsible for the failure to achieve biochemical and virological responses to IFN- α therapy in patients with chronic hepatitis C.

Interferons (IFNs) play a major role in the first steps of the response to acute viral infections, being mediators of the non-specific antiviral response that precedes the specific, immune-mediated, response (33). In addition to their antiviral effect, IFNs also have a variety of actions, e.g., immunomodulatory, cytostatic, and antitumor (2). The mechanism by which IFNs exert their antiviral action is complex and not fully elucidated. They may act at various stages of the viral cycle, but they mainly impair viral transcription and protein synthesis. Their effects are likely mediated by several biochemical pathways involving various proteins (23). Few of these proteins have been identified so far. The most widely investigated ones include the 2'-5' oligoadenylate synthetase (2-5OAS), the p68 kinase, and the Mx protein, cellular production of which is triggered by IFN- α in infected cells (7, 8, 31).

2-5OAS, when activated by double-stranded RNAs, polymerizes ATP into 2'-5'-linked oligomers of adenosine, re-

ferred to as 2'-5' oligoadenylate (2-5OA). The function of 2-5OA is to activate a latent endoribonuclease, responsible for degradation of viral and cellular RNAs. Measurement of 2-5OAS activity in serum, peripheral blood mononuclear cells (PBMC), or various tissues has been widely used as a surrogate marker for IFN- α induction. 2-5OAS activity is significantly induced at the acute stage of viral infections, a phenomenon likely due to virus-induced production of endogenous IFN- α (25, 28, 36), and it usually returns to basal levels when infection resolves. However, in some cases, the defense mechanisms of the host fail to eliminate the virus and chronic infection develops. This is the case with hepatitis C virus (HCV) infection, which becomes chronic in about 70% of the cases (1). Since 1986, IFN- α has been widely used in the treatment of chronic hepatitis C, on the basis of its antiviral, immunomodulatory, and antifibrotic properties (6). However, IFN- α treatment yields poor results in these patients. About 50% of them achieve biochemical response (normalization of alanine aminotransferase [ALT] activity) at treatment withdrawal, but sustained virological response (normal ALT activity and no evidence of HCV RNA by PCR [negative detection]) 6 months after treatment withdrawal is achieved in less than 15% of the patients (22, 26, 32). The reasons why so many patients are

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resistant to IFN- α are still unclear. For patients with chronic hepatitis B, another virus-related chronic liver disease responding to IFN- α therapy in about 30% of the cases, recent data suggested that a deficiency in the ability of 2-5OAS to be induced by exogenously administered IFN- α could account for the failure of IFN- α to clear the virus in more than two of three patients (15, 30). In patients with chronic hepatitis C, parenterally administered exogenous IFN- α significantly induces in vivo 2-5OAS activity in both serum and PBMC (5, 21). However, Giannelli et al. recently measured in vivo induction of 2-5OAS activity by IFN- α in the serum of patients with chronic hepatitis C and reported higher levels in biochemical responders than in nonresponders (5). These authors suggested that in vivo induction of 2-5OAS activity under IFN- α therapy might predict the response to treatment (5). In contrast, Kakumu et al. found no relationship between serum 2-5OAS activity and response to IFN- α (11). It must be stressed, however, that the significance of 2-5OAS activity measured in serum is uncertain.

The aim of the present study was to determine whether basal and in vivo and in vitro induced 2-5OAS activities, measured in PBMC of patients with chronic hepatitis C, may predict biochemical and virological responses to IFN- α treatment.

MATERIALS AND METHODS

Patients. 2-5OAS activity in PBMC and the antiviral effect of IFN- α were studied in 36 patients with chronic hepatitis C (27 men and 9 women; mean age, 44.7 ± 13.3 years). The diagnosis of chronic hepatitis C was based on the association of chronic increase in serum ALT activity, chronic active hepatitis on liver biopsy, positive serological markers for HCV infection (third-generation enzyme-linked immunosorbent assay and recombinant immunoblot assay; Ortho Diagnostic Systems, Raritan, N.J.), and evidence of serum HCV RNA detected by a highly sensitive reverse transcriptase nested PCR (20).

PBMC in the 36 patients were sampled before treatment for the study of 2-5OAS activity. Then, the patients received 3 MU of IFN- α -2a (Roferon-A; Roche Laboratories, Basel, Switzerland) three times a week subcutaneously. At month 3 of IFN- α therapy, PBMC were sampled 10 to 14 h after the last injection of the drug for the study of 2-5OAS activity. The latter delay has been shown to correspond with the peak of 2-5OAS induction by IFN- α after a single injection (3, 35). Patients who had normal serum ALT activity at month 3 had a supplemental 3-month treatment (overall treatment time, 6 months), whereas patients who still had elevated ALT activity at month 3 withdrew from therapy.

Parameters studied. 2-5OAS activity was measured in PBMC at month 0, i.e., before treatment (basal in vivo 2-5OAS activity) and at month 3 of IFN- α therapy, in order to study in vivo induction of 2-5OAS by IFN- α (IFN- α -induced in vivo 2-5OAS activity). The presence at month 0 and the induction at month 3 of two isoforms (p69 and p100) of 2-5OAS were qualitatively studied by Western blot (immunoblot) analysis in 20 of the 36 patients. On the other hand, PBMC sampled at month 0 were cultured for 24 h, either in the absence (basal in vitro 2-5OAS activity) or in the presence of IFN- α -2a, in order to study the pretreatment response of PBMC to IFN- α (IFN- α -induced in vitro 2-5OAS activity).

The antiviral effect of IFN- α -2a was assessed by measuring ALT activity in serum at month 3, month 6 (treatment withdrawal), and month 12 (6 months after treatment withdrawal) and by detection of serum HCV RNA by PCR at months 3 and 12.

Cell preparation. PBMC were isolated from 20 ml of heparinized venous blood by means of Ficoll-Hypaque density gradient centrifugation, washed twice in RPMI 1640, counted, and resuspended in 5 ml of RPMI 1640 supplemented with 20% heat-inactivated fetal calf serum, 0.3 mg of L-glutamine per ml, 0.5 μ g of amikacin per ml, and 0.25 μ g of vancomycin per ml. Of the total PBMC sample obtained at month 0 of IFN- α therapy, half was centrifuged, and the pellet was washed, frozen, and stored at -80°C for determination of basal in vivo 2-5OAS activity and Western blot analysis of 2-5OAS isoforms. The remaining cells were cultured for 24 h at 37°C in 9 ml of the same medium, either in the absence or in the presence of 500 U of IFN- α -2a per ml and then centrifuged. The pellet was washed, frozen, and stored at -80°C for the determination of basal and IFN- α -induced in vitro 2-5OAS activities. All the PBMC collected at month 3 of therapy were frozen and stored at -80°C for determination of IFN- α -induced in vivo 2-5OAS activity and Western blot analysis of 2-5OAS isoform induction.

Assay of 2-5OAS activity. 2-5OAS activity was assayed according to a modification of the published technique (9). For the preparation of cell extracts, cell pellets were suspended in 1 volume of homogenization buffer (10 mM Tris-HCl [pH 7.6], 10 mM KCl, 1 mM EDTA, 7 mM 2-mercaptoethanol, 1,000 U of aprotinin per ml, 0.2 mM phenylmethylsulfonyl fluoride). Extraction was performed in 1 volume of lysis buffer (homogenization buffer containing 2% Triton

X-100). Cell extracts were then diluted with immunoprecipitation buffer (20 mM Tris-HCl [pH 7.6], 50 mM KCl, 400 mM NaCl, 5 mM 2-mercaptoethanol, 1% Triton X-100, 0.2 mM phenylmethylsulfonyl fluoride, 100 U of aprotinin per ml, 20% [vol/vol] glycerol) to obtain a final KCl concentration of 75 mM and centrifuged at $1,500 \times g$ and at $12,000 \times g$. After the $12,000 \times g$ centrifugation, the supernatant was stored at -80°C .

Lymphocyte extracts (100 μ l) were added to aliquots (100 μ l) of poly(I)·poly(C)-Sephacrose previously equilibrated in a buffer containing 10 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; pH 7.6), 50 mM KCl, 2 mM magnesium acetate [$\text{Mg}(\text{OAc})_2$], 7 mM 2-mercaptoethanol, and 20% (vol/vol) glycerol. The binding of the 2-5OAS to poly(I)·poly(C)-Sephacrose was carried out in polystyrene tubes at 4°C for 2 h with gentle mixing. Proteins not bound to the poly(I)·poly(C)-Sephacrose were eliminated by washing three times in 5 ml of the same buffer. The supernatant obtained by centrifugation ($200 \times g$, 5 min) after each wash was removed by decanting.

The poly(I)·poly(C)-Sephacrose-bound synthetase fractions were resuspended in a total reaction mixture (300 μ l) containing 100 μ l of poly(I)·poly(C)-Sephacrose enzyme, 20 mM HEPES (pH 7.6), 50 mM KCl, 25 mM $\text{Mg}(\text{OAc})_2$, 7 mM 2-mercaptoethanol, 5 mM ATP, 10 mM creatine phosphate, 0.16 μ g of creatine kinase per ml, 0.1 mg of poly(I)·poly(C) per ml, and 1 ml of [^3H]ATP (1 mCi/ml). Incubation was for 17 to 18 h at 30°C and was terminated by heating at 90°C for 5 min. ^3H -labelled 2-5OA was purified by DEAE-cellulose chromatography as previously described (4) in a washing buffer containing 10 mM HEPES (pH 7.6), 50 mM KCl, and 2 mM $\text{Mg}(\text{OAc})_2$. Samples containing ^3H -labelled 2-5OA were applied to small columns (0.3 by 5 cm) of DEAE-cellulose equilibrated in the last washing buffer. After extensive washing with the same buffer (20 ml), the DEAE-cellulose columns containing 2-5OA were suspended in 3 ml of 0.3 N KOH, and the radioactivity of the entire sample was then measured after dilution into 10 ml of liquid scintillant cocktail for aqueous samples (Instagel, United Technologies, and Packard). The results were expressed as nanomoles of [^3H]AMP incorporated into 2-5OA per 10^6 lymphocytes per assay.

Western blot detection of the p69 and the p100 isoforms of 2-5OAS. p69 and p100 were measured by using specific mouse polyclonal antibodies and a Western blot assay. Cells were lysed in lysis buffer (20 mM Tris [pH 7.5], 50 mM KCl, 0.4 M NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 100 U of aprotinin per ml, 1 mM dithiothreitol, 1% Triton X-100, 20% [vol/vol] glycerol). Cell lysates were applied per lane of sodium dodecyl sulfate-polyacrylamide gels, and proteins were electrophoretically transferred to blots. 2-5OAS isoforms were detected by specific antibodies followed by ^{125}I -labelled sheep anti-mouse immunoglobulin G and autoradiography (13).

HCV RNA detection by PCR. PCR detection of HCV RNA in serum was performed according to the recently published procedure (20).

Statistical analysis. Results are presented as means $\pm$ standard error of the mean (SEM) or as percentages. Comparisons of quantitative parameters were made by means of the Mann-Whitney and the Wilcoxon nonparametric tests. For categorical parameters, the chi-square test and, when appropriate, the Fisher exact test were used.

RESULTS

Response to IFN- α therapy. The 36 patients were classified into five categories of response defined by serum ALT activity (elevated or within the normal range) at months 3, 6, and 12 and by the presence or absence of HCV RNA as determined by PCR at months 3 and 12. These five types of response were (i) early biochemical response, i.e., proportion of patients with normal serum ALT activity at month 3 (BR3); (ii) early virological response, i.e., proportion of patients with both normal ALT activity and negative HCV RNA detection at month 3 (VR3); (iii) biochemical response at treatment withdrawal, i.e., proportion of patients who had early biochemical response and still had normal ALT activity at month 6 (BR6); (iv) sustained biochemical response, i.e., proportion of patients who had normal serum ALT activity at month 6 and still had normal ALT activity at month 12 (BR12); (v) sustained virological response, i.e., proportion of patients who had both normal ALT activity and negative HCV RNA detection by PCR in serum at month 12 (VR12). The numbers of patients (and the percentages of the total number [36]) classified into each response category were as follows: BR3, 17 (47%); VR3, 8 (22%); BR6, 12 (33%); BR12, 5 (14%); VR12, 4 (11%).

2-5OAS activity in PBMC sampled before treatment. Mean $\pm$ SEM basal in vivo 2-5OAS activity in PBMC sampled at month 0 of IFN- α therapy was 4.41 ± 0.69 nmol/ 10^6 cells (range, 1.0 to 20.2) for the 36 patients with chronic hepatitis C.

Table 1 shows mean basal in vivo 2-5OAS activities according to the response group. No significant differences were found in basal in vivo 2-5OAS activities between responders and nonresponders in any response group (Table 1).

In vitro IFN- α induction of 2-5OAS activity in PBMC sampled before treatment. We tested the capacity of IFN- α to induce 2-5OAS activity in vitro in PBMC sampled at month 0 in the 36 patients. In vitro 2-5OAS activity was significantly induced by IFN- α (basal, 5.47 ± 1.15 nmol/10⁶ cells [range, 0.2 to 29.6]; IFN- α -induced, 13.87 ± 3.08 nmol/10⁶ cells [range, 0.3 to 74.5]; $P = 0.0008$). However, as shown in Table 1, IFN- α -induced/basal in vitro 2-5OAS activity ratios were not significantly different for responders and nonresponders in any response group.

In vivo induction of 2-5OAS activity by IFN- α therapy. In vivo 2-5OAS activity was significantly induced at month 3 of IFN- α therapy compared with the basal level (18.07 ± 2.74 [range, 1.0 to 72.6] and 4.41 ± 0.69 nmol/10⁶, respectively; $P = 0.0001$). However, neither IFN- α -induced in vivo 2-5OAS activity nor IFN- α -induced/basal in vivo 2-5OAS activity ratios were significantly different for responders and nonresponders in any response group (Table 1).

Qualitative analysis of in vivo induction of 2-5OAS activity by IFN- α therapy. Previous works have demonstrated the existence of at least three forms of 2-5OAS in cultured human cells and in PBMC with molecular masses of 40 to 46, 69, and 100 kDa (8, 34). By the use of specific polyclonal antibodies raised against human 69- and 100-kDa forms of 2-5OAS (referred to as p69 and p100, respectively), we investigated the expression of these forms of 2-5OAS in the PBMC of patients before and after 3 months of IFN- α therapy. Figure 1 shows a typical result for three patients. Basal 2-5OAS activities were quantitatively and qualitatively different among the 36 patients, and in some cases, no activity was detectable. Similarly, induction of p69 and p100 after 3 months of IFN- α therapy was not observed in all IFN- α -treated patients, and it was possible to determine whether or not p69 and p100 had been induced by IFN- α therapy.

The p69 isoform of 2-5OAS synthesizes long oligomeric forms of 2-5OAS that were shown to efficiently activate the endonuclease. In contrast, the p100 isoform synthesizes shorter oligomers that would not activate the enzyme (9). At month 3 of therapy, p69, which can thus be considered the active form of 2-5OAS, was induced, as demonstrated by Western blot analysis (Fig. 1), in 10 of 20 (50%) patients tested. Induction of the p69 isoform was evidenced in PBMC of responders, as well as in PBMC of nonresponders in the five response categories (Table 2). In the same way, induction of the p100 isoform was observed in the PBMC of 14 of 20 (70%) patients tested, including responders and nonresponders at the different end point times (Table 2). There was no significant difference in the rate of detection of either isoform between responders and nonresponders to IFN- α therapy in any of the five response groups (Table 2).

DISCUSSION

The results of the present study suggest that in patients with chronic hepatitis C, (i) basal in vivo 2-5OAS activity does not predict biochemical or virological response to IFN- α therapy; (ii) the ability of PBMC 2-5OAS activity to be induced by IFN- α does not predict biochemical or virological response to IFN- α therapy; (iii) response to IFN- α is not related to the level of in vivo induction of 2-5OAS activity by exogenously administered recombinant IFN- α ; and (iv) response to IFN- α is not related to the induction by IFN- α of the p69 isoform of

TABLE 1. 2-5OAS activities in PBMC of 36 patients with chronic hepatitis C according to the biochemical and virological responses to IFN- α therapy^a

Response group ^b	Basal in vivo 2-5OAS activity (nmol/10 ⁶ cells) ^c		IFN- α -induced/basal in vitro 2-5OAS activity ratio ^d		IFN- α -induced/basal in vivo 2-5OAS activity ratio ^e	
	Resp.	Nonresp.	Resp.	Nonresp.	Resp.	Nonresp.
Biochemical response at month 3 (BR3)	4.8 $\pm$ 0.8 (1.0–20.2)	4.1 $\pm$ 0.5 (1.0–11.8)	3.7 $\pm$ 0.8 (0.1–18.6)	3.5 $\pm$ 0.4 (0.3–9.1)	7.2 $\pm$ 1.3 (0.4–32.3)	9.5 $\pm$ 2.5 (0.2–68)
Virological response at month 3 (VR3)	7.5 $\pm$ 1.0 (1.0–20.2)	3.5 $\pm$ 0.5 (1.0–11.8)	4.7 $\pm$ 1.0 (0.3–18.6)	3.3 $\pm$ 0.5 (0.1–10.6)	3.7 $\pm$ 0.6 (0.4–12.6)	9.7 $\pm$ 2.2 (0.2–68)
Biochemical response at month 6 (BR6)	5.3 $\pm$ 1.0 (1.0–20.2)	3.9 $\pm$ 0.5 (1.0–11.8)	4.4 $\pm$ 0.9 (0.1–18.6)	3.2 $\pm$ 0.4 (0.3–9.1)	7.9 $\pm$ 1.5 (0.4–32.3)	8.7 $\pm$ 2.3 (0.2–68)
Biochemical response at month 12 (BR12)	4.4 $\pm$ 0.7 (1.0–9.4)	4.4 $\pm$ 0.7 (1.0–20.2)	3.5 $\pm$ 0.7 (0.3–10.6)	3.6 $\pm$ 0.5 (0.1–18.6)	5.9 $\pm$ 0.8 (0.4–11.2)	8.8 $\pm$ 2.2 (0.2–68)
Virological response at month 12 (VR12)	5.2 $\pm$ 0.7 (1.0–9.4)	4.3 $\pm$ 0.7 (1.0–20.2)	3.4 $\pm$ 0.8 (0.3–10.6)	3.6 $\pm$ 0.6 (0.1–18.6)	6.5 $\pm$ 0.9 (0.4–11.2)	8.6 $\pm$ 2.1 (0.2–68)

^a All data are means $\pm$ SEM, with ranges given in parentheses. All pairs of data (values for responders [Resp.] and nonresponders [Nonresp.] in a given column and a given row) are not significantly different.

^b BR, patients with normal serum ALT activity; VR, patients with normal serum ALT activity and negative HCV RNA detection by PCR.

^c PBMC sampled before treatment.

^d The values are ratios of in vitro IFN- α -induced 2-5OAS activities to those found in untreated PBMC. IFN- α treatment was at 500 U/ml for 24 h.

^e PBMC sampled at month 3 of IFN- α therapy. The values are ratios of in vivo levels of 2-5OAS activities in PBMC after 3 months of IFN- α therapy to those found before the start of the treatment.

FIG. 1. Induction of p69 and p100 isoforms of 2-5OAS in PBMC of patients with chronic hepatitis C after 3 months of IFN- α therapy. Extracts of PBMC from three patients (lanes 1, 2, and 3 [both panels]) before (all lanes c) and after (all lanes i) 3 months of IFN- α therapy were analyzed by Western blotting with specific polyclonal antibodies for p100 and p69 (panels α -p100 and α -p69, respectively). Lanes contained material from 5×10^5 cells. An autoradiograph is shown. Note: the polyclonal antibodies raised against the p69 form of 2-5OAS, cross-react with, in addition to p69, an 80-kDa protein (referred to as p80) whose identity is not yet clear (17).

2-5OAS, which has been shown to be the active form of the enzyme (9).

Recent data did not argue for the involvement of any deficiency of the 2-5OAS system in the pathogenesis of chronic hepatitis C (5, 21), in contrast with chronic hepatitis B, the development of which could be favored by a deficient response of 2-5OAS to endogenous IFN- α stimulation during acute infection (10, 24). As far as the role of 2-5OAS in the response of chronic hepatitis C to IFN- α therapy is concerned, the results of recent reports were to some extent contradictory. Giannelli et al. measured serum 2-5OAS activity in patients with chronic hepatitis C before IFN- α treatment and found no significant difference between biochemical responders and nonresponders at treatment withdrawal (5). In contrast, in vivo induction of serum 2-5OAS activity was significantly higher in responders than in nonresponders. These authors concluded that in vivo induction of 2-5OAS by exogenously administered IFN- α was an accurate marker for the antiviral

action of the drug (5). In contrast, Kakumu et al. found no relationship between 2-5OAS activity in serum and the response to IFN- α therapy in a small number of patients (11). However, these results might be questioned, because the significance of 2-5OAS activity as measured in serum is uncertain. Indeed, no explanations have been provided for the presence of this intracellular enzyme in the serum. It has been proposed that this presence may be due to degeneration or cellular lysis of lymphocytes (27). In addition, in chronic HCV infections, variations in serum 2-5OAS activity could reflect disease activity and hepatocellular injury rather than IFN- α induction, because 2-5OAS has been shown to be normally present in hepatocytes (16) and the livers of these patients often display extensive lymphocytic infiltration (12, 29). This suggests that results obtained by measuring 2-5OAS activity in serum should be considered cautiously. For this reason, we investigated 2-5OAS activity in PBMC, which can be considered a cellular model similar to hepatocytes. Our results can therefore be more easily extrapolated to hepatocytes, which are the main site of HCV replication and the main target of IFN- α in infected individuals. It should be noted, in addition, that 2-5OAS activity in PBMC was subjected to high interindividual variability in PBMC. This is the reason why induced/basal activity ratios were used for statistical analysis in this study instead of measured values.

Taken together, our results suggest that a failure of the 2-5OAS system to be induced by IFN- α , which could be genetically determined, is unlikely to be responsible for the lack of biochemical and virological responses to IFN- α therapy in patients with chronic hepatitis C. In other words, the lack of response to IFN- α therapy does not seem to be related to an inherited or acquired inability of the host to synthesize appropriate antiviral molecules under the influence of exogenously administered IFN- α . It must be stressed, however, that our data were limited to a single component of the IFN- α system. Information concerning the induction of other IFN- α -responsive genes and IFN- α receptor levels in PBMC would clarify the significance of the observation regarding 2-5OAS. Conversely, viral parameters are likely to play a predominant role in the sensitivity of patients with chronic hepatitis C to IFN- α treatment. Several virological parameters were recently reported to be significantly associated with sustained virological response to IFN- α . These factors included HCV genotype, viral load, the presence in serum of anti-HCV core immunoglobulin M antibodies, a parameter significantly related to HCV genotype, and, possibly, the level of genetic heterogeneity of the strain (14, 17, 18, 19, 22). Future studies on the mechanisms of resistance of chronic hepatitis C to IFN- α therapy should focus on these parameters, especially the possible role of specific mutations occurring in the genome as a result of its quasispecies distribution and of the selection by IFN- α of resistant viral populations.

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TABLE 2. In vivo induction of the p69 and p100 isoforms of the PBMC 2-5OAS by IFN- α treatment, according to the biochemical and virological responses to IFN- α therapy in 36 patients with chronic hepatitis C

Response group ^a	Induction of indicated isoform of 2-5OAS (no. of patients with induction/no. of patients in response group) ^b			
	p69		p100	
	Resp.	Nonresp.	Resp.	Nonresp.
Biochemical response at month 3 (BR3)	4/9	6/11	6/9	8/11
Virological response at month 3 (VR3)	3/4	7/16	3/4	11/16
Biochemical response at month 6 (BR6)	2/5	8/15	3/5	11/15
Biochemical response at month 12 (BR12)	2/4	8/16	2/4	12/16
Virological response at month 12 (VR12)	1/3	9/17	1/3	13/17

^a BR, patients with normal serum ALT activity; VR, patients with normal serum ALT activity and negative HCV RNA detection by PCR.

^b Only 20 patients among the 36 included in the study were tested for p69 and p100 isoforms of 2-5OAS by Western blot analysis. Resp., responders; Nonresp., nonresponders.

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