Alpha interferon combined with ribavirin potentiates proliferative suppression but not cytokine production in mitogenically stimulated human lymphocytes

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Received 26 May 1999; accepted 9 August 2000

Abstract

The improved clinical outcome observed among patients with hepatitis C treated with the combination of alpha interferon (IFN) and ribavirin (RBV) is presumed to result from immunomodulation and viral inhibition. However, the impact of the drug combination upon lymphocyte activity is unknown. The present study evaluated the effects of IFN and RBV, singly and in combination, upon proliferation, cell cycle sensitivity and cytokine elaboration following PHA stimulation of lymphocytes. Two formulations of IFN, interferon-a-2b (IFN-2b) and interferon-a-con-1 (CIFN), were included. Titration of each drug over a wide range of concentrations showed dose dependent proliferative suppression without cytotoxicity. Proliferation was suppressed 57–99% (P < 0.001) by IFN-2b (10^5–10^7 IU/ml), 41–74% (P < 0.001) by CIFN (1.5–150 ng/ml), and 10–94% (P < 0.001) by RBV (0.5–50 μg/ml). Isobologram analysis showed that the interaction between IFN-2b and RBV on proliferative suppression was additive. In contrast, the interaction between CIFN and RBV was weakly antagonistic. Proliferative suppression by both the IFNs was cell cycle restricted. IFN-2b and CIFN added at the onset of PHA stimulation (G0/G1) versus 24 h later (S phase) inhibited proliferation by 50 versus 5%, respectively (P < 0.05). The onset of IFN resistance correlated with a 50% reduction (P < 0.05) in IFN receptors on the cell surface. In contrast, RBV caused equivalent proliferative suppression (P = NS) when added at any time during PHA activation. Cytokine secretion after 24 h of PHA stimulation showed that IFN-2b versus CIFN increased the secretion of IL2, TNF and gamma IFN by 4.5-, 4.1- and 8.3-fold (P < 0.005) versus 1-, 1.9- and 1.9-fold (P < 0.05), respectively, above control levels. Neither IFN affected IL10 secretion. RBV, singly and in combination with IFN, had no impact on cytokine expression (P = NS). This study identifies several potential mechanisms by which the combination of IFN and RBV may exert a more potent effect upon cellular immunity than either agent alone and shows that different formulations of IFN may have non-identical effects upon lymphocyte responses. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Hepatitis C; Interferon; Ribavirin; Immunomodulation; Cell cycle sensitivity; Interferon receptor

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PH: S0166-3542(00)00120-0
1. Introduction

Interferon-alpha (IFN) is the primary therapy for patients with chronic hepatitis C virus (HCV). Unfortunately, less than half of all patients treated with IFN achieve either a biochemical or virologic response and the great majority of these individuals relapse following discontinuation of therapy (Davis et al., 1989; DiBisceglie et al., 1989; Causse et al., 1991; Marcellin et al., 1991; Lindsay, 1997). The use of ribavirin (RBV) in combination with IFN has significantly increased the number of patients demonstrating clinical improvement although permanent resolution of viral infection is rarely observed for most HCV genotypes (Davis et al., 1998; McHutchinson et al., 1998; Poynard et al., 1998; Reichard et al., 1998).

Functional insufficiency among cytotoxic T-lymphocytes is conceptualized as one mechanism to explain HCV persistence in vivo (Chang, 1998). In that context, any pharmacologic agent producing a clinical benefit is anticipated to be mediated, at least in part, by immune modulation. The mechanisms by which IFN/RBV combination therapy improve clinical response in patients with chronic HCV is poorly understood. And while there is ample justification to anticipate that both drugs are immunomodulatory, the specific impact of the combination of drugs upon T-cell function has not been rigorously examined. Interferon alpha is a cytokine with anti-viral and immunomodulatory activity (Nelson and Borden, 1989; Peters, 1989; Gutterman, 1994). Ribavirin is a nucleoside analog with in vitro and in vivo virustatic activity against a spectrum of DNA and RNA viruses and recent studies suggest that it may also possess immunomodulatory activity (Patterson and Fernandez-Larsson, 1990; Foster, 1997). Use of RBV monotherapy in patients with chronic HCV markedly reduced serum liver enzyme levels but did not change circulating viral titers. RBV has been shown to alter cytokine secretion profiles during the activation of particular lymphoid populations (Hultren et al., 1988; Bodenheimer et al., 1997).

The present in vitro study was conducted to compare the impact of IFN and RBV, singly and in combination, upon lymphocyte proliferation and cytokine elaboration following in vitro stimulation with phytohaemagglutinin (PHA). Two commercial sources of IFN, both of which are used for the clinical management of HCV disease, were included in the study. The results showed the IFN/RBV combination caused significant proliferative suppression among T-cells, enhanced the secretion of TH1 cytokines and displayed differential cell cycle restrictions which may optimize the consequences of multidrug exposure upon lymphoid cells.

2. Materials and methods

2.1. Cell proliferation

Peripheral blood was collected into ACD tubes following venipuncture of healthy volunteers and lymphocytes (PBL) isolated by Ficoll-Hypaque density sedimentation. PBLs were propagated in RPMI 1640 with 10% fetal bovine serum at 37°C with 5% CO₂ in air. Proliferation was measured by cultivating 200 μl of 2 × 10⁶ cells/ml into 96-well microtiter plates and stimulating with 5 μg/ml phytohaemagglutinin-L (PHA) (Pharmacia Biotech, Piscataway, NJ) for 72 h. During the last 12 h of culture, cells were pulsed with 1 μCi/ml [³H]thymidine. Nuclear incorporation of isotope was quantitated and data are expressed in counts per minute (cpm).

The effect of various concentrations of interferon-a-2b (IFN-2b; Schering-Plough, Kenilworth, NJ), interferon-a-con-1 (CIFN; Amgen, Thousand Oaks, CA) and ribavirin (Schering-Plough, Kenilworth, NJ) on lymphocyte proliferation was assessed by adding variable concentrations of these agents to the culture media at the onset of culture. Stock drug concentrations were diluted in RPMI and sterilized by filtration through 0.22-μm filters. The maximum concentration of each drug used in the initial experiments was less than the maximum clinical dose recommended by the manufacturer. The manufacturer’s recommended doses for IFN-2b, CIFN and RBV are 3 million IU/ml, 1–9 μg/ml and 15 mg/kg per day, respectively. Circulating levels of IFN-2b, CIFN and RBV are reported in
the range of 300 IU/ml, 1 ng/ml and 1 μg/ml, respectively. The specific activity for IFN-2b is $2.6 \times 10^8$ IU/mg. The maximum concentration tested for IFN-2b, CIFN and RBV was $10^7$ IU/ml, 150 ng/ml and 50 μg/ml, respectively. Each drug was serially diluted such that ten concentrations were tested per drug. Thus, all drugs were tested at physiologic and non-physiologic concentrations. The impact of various combinations of IFN and RBV upon proliferation was then evaluated. Results on the nature of drug:drug interaction were evaluated by isobologram analysis as described below. Cell viability at the end of culture was measured by trypan blue exclusion and exceeded 90%.

Addition of drugs at various times during PHA activation has been used to evaluate cell cycle restrictions among immunosuppressive drugs such as cyclosporine (Kimball et al., 1990). The impact of the cell cycle upon drug sensitivity was tested by adding IFN or RBV to PHA stimulated PBLs at the onset of PHA activation (G0/G1) and 24 h after PHA stimulation (S phase) (Kimball et al., 1990; Kimball and Rhodes, 1999). Proliferation was quantitated as described above.

2.2. Isobologram analysis

Isobologram analysis is a technique which evaluates whether the interaction between two or more drugs is synergistic, additive or antagonistic (Colombani et al., 1989; Kimball et al., 1990). The analysis was performed by assessing the proliferative response of PHA stimulated PBLs to ten concentrations of two drugs when administered simultaneously. The fractional inhibitory concentration (FIC) for each drug combination, at 50% inhibitory concentration for example, can be calculated using the following formula:

$$\text{FIC} = \frac{\text{IC}_{50}(\text{drug A combined})}{\text{IC}_{50}(\text{drug A alone})} + \frac{\text{IC}_{50}(\text{drug B combined})}{\text{IC}_{50}(\text{drug B alone})}$$

The FIC index for each mixture is plotted with a line of unity drawn between FIC = 1 for drug A and FIC = 1 for drug B. In such an analysis, curves falling below the line of unity indicate synergy, curves on the line indicate an additive reaction, and curves above the line indicate that the drug combination is antagonistic.

2.3. Quantitation of interferon receptors

The expression of interferon alpha receptor (IF-NaR) on the lymphocyte cell surface was quantitated by indirect immunofluorescence using a 1024-channel FACScan (BD, St. Louis, MO). PBLs were cultivated with and without PHA for 0, 1 and 24 h. Cells were washed extensively and incubated with mouse anti-human alpha interferon receptor (IFNaR3 was the kind gift of Dr Colamonici, University of Illinois) at 4°C for 1 h, then FITC conjugated goat anti-mouse Ig (Chemicon, Temacula, CA, FC fragment) at 4°C for 1 h in the dark. Forward and side scatter parameters were gated to the lymphocyte compartment. Non-specific binding was excluded by use of irrelevant primary antibody. The percentage of cells expressing receptor as well as the density of receptor on the cell surface was determined. The density of cell surface IFNaR was expressed as median excitation soluble fluorescent (MESF) units following standardization with fluorescent calibration beads (BD) and analysis using Quantum 24/QuickCal Software (FCSC, San Juan, Puerto Rico).

2.4. Cytokine secretion

PBLs ($2 \times 10^6$ cells/ml) were stimulated with PHA for 24 h. Single and multidrug combinations of IFN-2b, CIFN and RBV were added to the culture media at the onset of PHA activation. Drug concentrations were selected on the basis of proliferative inhibition as shown in Figs. 1 and 2. Culture supernatants were harvested, spun and analyzed for IL2, IL10, tumor necrosis factor (TNF) and gamma interferon (g-IFN) by ELISA (R&D Systems, Minneapolis, MN).

2.5. Statistics

All experiments were performed three to seven times. Data were expressed as means ± S.D. and
analyzed by two-tailed unpaired t-test, chi-square and one-way ANOVA using Graphpad Prism Software (San Diego, CA). A P-value of ≤ 0.05 was considered significant.

3. Results

3.1. Effect of IFN and RBV, singly and in combination, on lymphocyte proliferation

The effect of IFN-2b, CIFN and RBV upon PHA stimulated proliferation was initially evaluated using ten concentrations of each drug which encompassed physiologic and non-physiologic levels (Figs. 1 and 2). Each individual drug suppressed proliferation in a dose dependent manner (Fig. 1). Viability at the end of the experiments exceeded 90% as assessed by trypan blue exclusion. IFN-2b at doses of $10^5$, $10^6$ and $10^7$ IU/ml reduced proliferation relative to drug-free cultures by 57% ($P < 0.0001$), 78% ($P < 0.0001$) and 99% ($P < 0.0001$), respectively. CIFN at doses of 1.5, 15 and 150 ng/ml reduced proliferation by 41% ($P = 0.0002$), 60% ($P < 0.0001$) and 74% ($P < 0.0001$), respectively. Lastly, RBV at doses of 0.5, 5 or 50 µg/ml reduced proliferation by 10.3% ($P = 0.0009$), 62.9% ($P < 0.0001$) and 94% ($P < 0.0001$), respectively.

Isobologram analysis is an analytical method to mathematically describe the nature of drug:drug interactions as additive, antagonistic or synergistic (Colombani et al., 1989; Kimball et al., 1990). In this series of experiments, the maximum individual drug concentration was selected to reduce proliferation by ≤ 50%. The maximum concentration of each two drug combination was serially and simultaneously titrated ten times so that drug levels ranged from physiologic to sub-physiologic. The results of combined IFN-2b/RBV and CIFN/RBV treatment upon proliferation are shown (Fig. 2). The data generated in the proliferative curves were used in the isobologram calculations. Treatment of lymphocytes with combinations of IFN-2b and RBV appeared to produce an additive anti-proliferative response (Fig. 2A). Isobologram analysis demonstrated an FIC ratio between 0.8 and 1.0 (Fig. 3) which confirmed that the immunosuppressive effects of these agents when used in combination are additive. In contrast, the combination of CIFN/RBV appeared to exert an antagonistic effect upon proliferative suppression (Fig. 2B). Isobologram analysis supported this observation and demonstrated an FIC ratio of 1.5 indicating that the relationship between the two drugs is weakly antagonist (Fig. 3), that is, that the proliferative suppression resulting from the combination was less than that observed with either drug alone.

Fig. 1. The effects of two commercial sources of IFN and RBV upon lymphocyte proliferation. Cells were stimulated with PHA and incubated with a wide range of concentrations of each drug for 72 h. Proliferation was assessed by measuring the amount of [³H]thymidine incorporated during the last 12 h of culture and expressed as the mean cpm ± S.D. from replicate experiments. (A) IFN-a-2b; (B) CIFN; (C) RBV.
3.2. Effect of cell cycle upon proliferative sensitivity to IFN and RBV

The ability of IFN and RBV to suppress proliferation at various stages of the cell cycle was evaluated by adding the drugs at the onset of PHA activation (G0/G1) or 24 h after PHA activation (S phase). RBV treatment resulted in equivalent proliferative suppression when added at the onset of cultivation or 24 h later (Table 1). In contrast, both IFN-2b and CIFN inhibited proliferation only when added during the initial hours of PHA stimulation. Thus, the proliferative suppression caused by the IFNs was restricted to G0/G1 phase of the cell cycle whereas the action of RBV was cell cycle independent.

To determine if this effect was secondary to downregulation of surface expression of the alpha interferon receptor during S phase of the cell cycle, the frequency and density of receptors was quantitated at the onset and 24 h after PHA stimulation. The percentage of cells expressing IFNaR3 as well as the relative surface density of IFNaR3 was equivalent among cells prior to or within 1 h of PHA stimulation (Fig. 4). In contrast, both the number of cells with IFNaR and the density of receptors were significantly reduced \( (P < 0.05) \) after 24-h PHA activation when cells are in S phase of the cell cycle.

3.3. Impact of IFN and RBV upon cytokine elaboration

The impact of IFN and RBV when utilized singly and in combination upon cytokine secre-
Table 1
Proliferative suppression following immediate or delayed addition of drugs during PHA stimulation

<table>
<thead>
<tr>
<th>Agent added</th>
<th>Immediately After 24 h (%)</th>
<th>P-value</th>
<th>After 24 h (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RBV (mg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td>51.3 ± 8.3</td>
<td>48 ± 22</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>83.4 ± 1.9</td>
<td>88.9 ± 5.9</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td><strong>IFN-2b (× 10^2 IU/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>47.4 ± 24.3</td>
<td>2.3 ± 2.3</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>53.4 ± 20.6</td>
<td>3.8 ± 3.4</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td><strong>CIFN (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.2</td>
<td>36.9 ± 1.8</td>
<td>9.6 ± 8.7</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>70.4 ± 9</td>
<td>4.7 ± 4.7</td>
<td>0.006</td>
<td></td>
</tr>
</tbody>
</table>

* Two concentrations of each drug were added to PHA stimulated PBLs at the onset of cultivation (G0: G1 phase) or 24 h after the start of culture (S phase). Proliferation was measured by tritiated thymidine incorporation and data are expressed as the percent suppression relative to drug-free cultures.

Table 2
Impact of IFN-2b and CIFN upon cytokine elaboration

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>IFN-2b (5 × 10^2 IU/ml)</th>
<th>CIFN (5 ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Without RBV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL2</td>
<td>4.5 ± 2.2*</td>
<td>1 ± 0.4***</td>
</tr>
<tr>
<td>IL10</td>
<td>1 ± 1***</td>
<td>0.9 ± 0.5***</td>
</tr>
<tr>
<td>TNF</td>
<td>4.1 ± 1.9***</td>
<td>1.9 ± 0.8*</td>
</tr>
<tr>
<td>g-IFN</td>
<td>8.3 ± 0.5**</td>
<td>1.9 ± 0.6*</td>
</tr>
<tr>
<td><strong>B. With 833 ng/ml RBV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL2</td>
<td>4.9 ± 3.9*</td>
<td>1.9 ± 0.9***</td>
</tr>
<tr>
<td>IL10</td>
<td>1.2 ± 1.2***</td>
<td>1.2 ± 0.5***</td>
</tr>
<tr>
<td>TNF</td>
<td>4.5 ± 1.8***</td>
<td>3 ± 0.3***</td>
</tr>
<tr>
<td>g-IFN</td>
<td>9.6 ± 3.1***</td>
<td>2.7 ± 1.1**</td>
</tr>
</tbody>
</table>

*Cytokine content was measured in the supernatants of PBLs stimulated with PHA for 24 h and cocultivated with single or multi-drug regimens. Data are expressed as the fold-increase in cytokine content relative to the level found in drug-free PHA stimulated cultures. Statistical evaluation compared drug-treated to drug-free cytokine levels. 

* P < 0.05.

** P < 0.005.

*** P = NS.

Incubation of PHA stimulated PBLs with 500 IU/ml IFN-2b increased the release of IL2, TNF and g-IFN by 4.5 ± 2.2 (P < 0.05), 4.1 ± 1.9 (P < 0.005) and 8.3 ± 0.5 fold (P < 0.005), respectively, relative to that observed with PHA stimulation alone. Incubation with 5 ng/ml CIFN increased the release of TNF and g-IFN by 1.9 ± 0.8 (P < 0.05) and 1.9 ± 0.6 (P < 0.05), respectively, above that of PHA alone. In contrast, treatment with CIFN did not increase IL2 secretion and TNF and g-IFN levels were significantly lower (P < 0.05) than with IFN-2b. Neither IFN-2b nor CIFN affected the secretion of IL10. In contrast, incubation of PHA stimulated PBLs with 833 ng/ml RBV did not alter cytokine release. The ratios of IL2 IL10, TNF and g-IFN secretion in...
the presence of RBV relative to drug-free cultures were $0.6 \pm 0.6$ (NS), $0.8 \pm 0.2$ (NS), $0.8 \pm 0.1$ (NS) and $0.5 \pm 0.4$ (NS), respectively. Combined treatment of PHA stimulated cells with RBV and IFN did not significantly affect the release of cytokines when compared to levels secreted by either IFN-2b or CIFN alone (Table 2). Secretion of IL2, TNF and $\gamma$-IFN was significantly lower ($P < 0.05$) with the CIFN/RBV combination that with the IFN-2b/RBV combination.

4. Discussion

To date, treatment with IFN is the most effective therapy for patients with HCV. IFN demonstrates both anti-viral and immunomodulatory properties. The anti-viral action of IFN has been attributed to the ability of this agent to stimulate the generation of cytotoxic T-cells as well as inhibit 2,5-oligo-adenylate synthetase, an intracellular enzyme necessary for viral transcription and protein synthesis within infected cells. The immunomodulatory action is less well understood but it appears that administration of IFN causes an alteration in the types of cytokines produced by T-lymphocytes. It is speculated that this change in the cytokine milieu contributes to viral regulation and may alter inflammatory responses of the host (Pestka et al., 1987; Peters, 1989; Gutterman, 1994; Foster, 1997; Chang, 1998; Mizukoshi et al., 1998).

Ribavirin was initially utilized for the treatment of chronic HCV because it demonstrates in vitro virustatic activity against a number of DNA and RNA viruses. RBV is believed to act by inhibiting inositol monophosphate dehydrogenase which depletes intracellular stores of guanine triphosphate and reduces DNA and RNA synthesis (Patterson and Fernandez-Larsson, 1990). Other studies implicate immunomodulatory activity by RBV. When patients with chronic HCV were treated with RBV a marked reduction in serum ALT was observed without any effect on HCV viral titers (Marcellin et al., 1991; Reichard et al., 1998). RBV has also been shown to alter the types of cytokines secreted from T-lymphocytes isolated from transgenic mice immunized with hepatitis B and to affect macrophage and neutrophil function (Hultgren et al., 1988; Bodenheimer et al., 1997; Ning et al., 1998; Steele et al., 1998).

The manner by which IFN and RBV interact to achieve a biologic response is not clear and, in fact, the effects of the combined agents upon in vitro parameters have not been explored. The present in vitro study was performed to compare the impact of IFN and RBV tested singly and in combination upon specific lymphocyte cell parameters including proliferation, cell cycle sensitivity and cytokine elaboration. Because several types of IFNs are used to treat patients with chronic HCV, we included two different sources of IFN in the study. The demonstration that the in vitro effects of IFN-2b and CIFN were non-identical was unexpected. Of particular interest was the demonstration of qualitative differences in the impact of the two IFNs upon T-cell proliferation. Specifically, the combination of IFN-2b/RBV had an additive effect upon proliferative suppression whereas the combination of CIFN/RBV was weakly antagonistic. It is tempting to extrapolate these in vitro findings to a clinical scenario and speculate that the IFN-2b/RBV combination may be more efficacious in the medical management of HCV than CIFN/RBV. However, this study does not address that issue which can only be evaluated by clinical comparison.

This study clearly demonstrated that each IFN and RBV, when tested as single agents or in combination, potently inhibited T-cell proliferation. This study is consistent with previous demonstrations of anti-proliferative effects of alpha interferon (Nelson and Borden, 1989; Gutterman, 1994; Foster, 1997; Koziel, 1999) and corroborates the observation of Hultgren et al. (1998) that RBV has anti-proliferative effects. However, this is the first study to demonstrate that proliferative suppression is enhanced by the combination of IFN and RBV and that different formulations of IFN exhibit differential immunosuppressive potencies. How an anti-proliferative effect in vitro corresponds to reduced viral load in vivo is not known but underscores the complexity of the pharmacologic actions of these two agents which probably simultaneously promote anti-viral, anti-immune and anti-metabolic activities. In
the conceptualized model of HCV disease, pharmacologically induced immunosuppression would seem to be undesirable. However, results from this and other studies showing anti-proliferative effects of these agents may warrant a revision of our perspectives upon immune involvement in HCV disease and the impact of combination therapy in HCV management. If T-cells expand or respond inappropriately to HCV infected tissues, it is very likely that the lymphocytes could contribute substantially to hepatocellular damage which is the hallmark of HCV disease progression. Although IFN and RBV inhibit viral replication, they may provide additional clinical benefit by suppressing excessive immune reactions of the host, thus reducing the immunologic assault upon virally infected hepatic tissue. In that context, use of two different immunosuppressive drugs might enhance the immunosuppression and reduce the undesirable side-effects of either agent alone.

Although quantitatively dissimilar, both IFNs produced a qualitatively similar impact upon cytokine elaboration. Both IFN and CIFN induced a pro-inflammatory TH1-like profile and both caused an overproduction of those cytokines relative to drug-free cultures. In contrast, RBV did not alter cytokine levels or composition when used as a single agent or in combination with IFN. Other studies have reported production of TH1 cytokines in the presence of IFN, but results with RBV are contradictory and range from no impact upon cytokine production to induction of TH1 or TH2 profiles (Hultren et al., 1988; Kimball et al., 1996; Martin et al., 1998; Tam et al., 1999). Given the wide range of experimental conditions and assay systems used in previous studies, it is not surprising that conflicting results have been observed. The advantage of the present study was that the effects of IFN and RBV as well as the combinations were compared under identical conditions. The finding of overproduction of TH1 cytokines in the face of IFN (but not RBV) induced proliferative suppression suggests that, in this case, proliferative reduction is not exclusively linked to an abnormal cytokine milieu. The data imply that immunosuppression by IFN and RBV are mediated by different mechanisms which may contribute to the improved clinical outcome when the drugs are used together.

It is well known that the effects of IFN are mediated by binding to specific receptors on the cell surface (Mizukoshi et al., 1998). The present study demonstrated that the number of cells with alpha interferon receptor and the density of those receptors were maximal during G0 or G0/G1 phase of the cell cycle. However as T-cells entered the replicative phase of the cell cycle, the number and density of receptors on the cell surface declined significantly. As a consequence, the anti-proliferative effect of IFN was abolished when added to cells in S phase. In contrast, the anti-proliferative effect of RBV was independent of the phase of the cell cycle. This observation provides another potentially useful sequela of IFN/RBV combination as compared to IFN monotherapy in the treatment of chronic HCV. Since the anti-proliferative effects of IFN appeared cell cycle restricted, any immune stimulation occurring during clinical treatment might stimulate T-cell proliferation and reduce interferon receptor content thus rendering a patient IFN-insensitive during the course of an infection. Such a stimulus might be a bacterial or viral infection or possibly even the development of new HCV quasispecies which are known to develop during the course of IFN therapy (Kanazawa et al., 1994; Mizokami et al., 1994; Gonzalez-Peralta et al., 1996). Since the immunomodulatory effects of RBV would not be affected by immune activation, the use of IFN/RBV combination therapy guarantees that some level of immune attenuation is always maintained despite any exogenous immune stimulus which might occur during the course of treatment.

Given the limited success of our current therapies for chronic HCV, the search for more effective treatments will no doubt continue. In the absence of a proven anti-viral agent against HCV, drug combinations which modulate the immune response, such as IFN and RBV, will continue to dominate the therapy of this disease. The present in vitro studies have demonstrated that isobologram analysis can be a useful tool to evaluate the interactions between drug combinations which may be of clinical interest. The relevance of in vitro studies to predict clinical outcome can only be determined by parallel in vivo studies.
Acknowledgements

This study was supported by ROI NR04395 from the National Institutes of Health.

References


