Differences in Viral Dynamics between Genotypes 1 and 2 of Hepatitis C Virus

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Many studies have shown that patients infected with hepatitis C virus (HCV) of genotype 2 have better response to interferon (IFN)-α treatment than genotype 1 patients; however, the mechanisms responsible for this difference are not understood. In this study, viral dynamics during high-dose IFN induction treatment were compared between the genotypes. Patients in each group received 10 MU of IFN-α2b for 14 days, and HCV RNA levels were frequently determined. Nonlinear fitting, both individually for each patient and using a mixed-effects approach, of the viral kinetic data to a mathematical model of the IFN effect on HCV infection was performed. The antiviral effectiveness of IFN in blocking virus production, the free virion clearance rate, and the HCV-infected cell death rate were all significantly higher for genotype 2 patients than for genotype 1 patients. Thus, the better response rate of patients infected with HCV genotype 2 is multifactorial. This is the first finding of a difference in viral dynamics between subtypes of the same virus and demonstrates the importance of subtype-specific virus-host-drug interactions.

Chronic hepatitis C virus (HCV) infection causes an indolent hepatic disease that may, over 1–4 decades, progress to cirrhosis, decompensated liver disease, and hepatocellular carcinoma [1, 2]. It is has a high prevalence, of 1%–3%, around the world, and despite a decline in new HCV infections, it has been estimated that the need for hepatic transplantation for HCV due to its outcomes will increase 5- to 7-fold in the next 20 years unless therapy becomes more effective [3]. Interferon (IFN)-α has been the mainstay of HCV therapy, although sustained virological clearance occurs in only 7%–20% of patients treated for 1 year [4]. The addition of the nucleotide analogue ribavirin to IFN therapy has increased sustained viral response to 30%–40% [5, 6]. Therapeutic response to both IFN monotherapy [7, 8] and combination therapy [5, 6] has been shown to be significantly influenced by the genotype of HCV infecting the patient. For example, sustained virological response occurs in 65% of patients receiving IFN-α2b and ribavirin and infected with viral genotypes 2 and 3 but in only 30% of patients infected with genotype 1 [5, 6]. The reason for these dramatic differences in viral response rates between these closely related virus strains is unknown.

We have recently described the early viral kinetics decline in response to daily high-dose IFN therapy in patients infected with HCV of genotypes 1a and 1b and have shown that virus levels decline in a biphasic manner [9–11]. The first phase of decline is rapid, accounting for a 0.5–2 log decline in HCV RNA levels within 48 h of initiating therapy, with the extent of decline being highly dose dependent. After this rapid decline, a slower second phase of HCV RNA decline ensues. The rate of viral decline during this phase has been shown to be highly variable between patients and not dose dependent. Furthermore, the second-phase slope, which we found to be inversely correlated with baseline virus load, is a good predictor of serum virus becoming undetectable after 3 months of therapy [9]. Using mathematical models, we have shown that the biphasic decline can be explained by IFN partially blocking the production and or release of virions [9]. The slope of the first phase was attributed to the clearance of free virions, whereas the second-phase slope can be attributed to the loss of HCV-infected cells, with both slopes depending also on the IFN effectiveness in blocking production. Blocking of de novo infection by IFN is a possible, but not necessary, additional effect...
and does not considerably change the biphasic decline if the major IFN effect is blocking production [9].

In the present study, using these same mathematical principles, we analyze and compare the early viral dynamics following initiation of IFN therapy (10 MU daily) in patients infected with genotype 1 with that observed in patients infected with genotype 2 virus. We show that the greater viral response in genotype 2-infected patients is due to multiple factors, including higher drug effectiveness, a faster free virion clearance rate, and possibly an enhanced immunological response. This is the first finding of a difference in viral dynamics between two subtypes of the same virus.

**Methods**

**Patients.** Patients seen at the University of Illinois, Chicago, Liver Center, who had HCV confirmed by a positive HCV RNA level and an elevated aminotransaminase level, were asked to be screened for the study. The study was approved by the University of Illinois Institutional Review Board and the Clinical Resource Scientific Committee. To be enrolled in the study, patients had to have either genotype 1a or 1b or genotype 2a or 2b infection, diagnosed using the genotyping method of Okamoto et al. [12] in accordance to the genotype nomenclature of Simmonds et al. [13]. We did not further separate the patients into the subtypes of each genotype, because elsewhere [9] we did not find a difference between the subtypes (1a and 1b) of genotype 1, and here 8/9 genotype 2 patients are of subtype 2b. Patients had a complete history and physical and were excluded if they had evidence of decompensated liver disease or a history of ongoing alcohol intake or illicit drug use. Other standard inclusion and exclusion criteria for IFN therapy were used [9]. If patients had not had a liver biopsy in the 2 years prior to the study, they were asked to undergo a percutaneous biopsy as part of standard practice. These biopsies and those performed in the last 2 years were reviewed in a blind fashion by one of the authors (T.J.L.) and were rated for histological activity using the scoring system of Knodell et al. [14]. The patients with genotype 1 or genotype 2 infection included in this study were enrolled over the same time period, and the assays were used to measure HCV RNA levels were the same. Results for the genotype 1–infected patients have been published elsewhere [9].

**Protocol and samples.** Patients were admitted to the Clinical Resource Center, and a small intercatheter was placed for blood draw. At 8:00 AM patients were injected subcutaneously with 10 MU IFN-a2b (Schering Plough, Kenilworth, NJ). Blood was drawn at 0, 2, 4, 7, 10, 14, 19, and 24 h after the first injection. Serum was separated within 4 h of venipuncture, aliquoted, and stored at −70°C. At 8:00 AM on each of the following 13 days, the patients injected themselves with 10 MU of IFN. On the second day, blood was withdrawn at 5, 10, and 24 h and on days 3–5, 7, 9, 11, 12, and 14 before IFN injection. After 14 days, the patients received a maintenance treatment of 5 MU IFN daily for 6 months (results not shown).

HCV RNA quantification. HCV RNA levels were quantified by the branched-DNA method (Quantiplex 2.0, Chiron, Emoryville, CA) and by a polymerase chain reaction (PCR) method (Superquant, National Genetic Institute, Los Angeles). Criteria for the use of the results from each of these assays were applied as described elsewhere [9].

**Mathematical model.** Data were analyzed using our previously published mathematical model of the effect of IFN on HCV infection dynamics [9]. The model’s differential equations are:

\[\frac{dT}{dt} = s - dT - (1 - \eta) \beta VT,\]  
\[\frac{dI}{dt} = (1 - \eta) \beta VT - dI,\]  
\[\frac{dV}{dt} = (1 - \eta) pT - cV,\]

where \(T\) represents the number of target cells, \(I\) represents the number of productively infected cells, and \(V\) is the virus load. Target cells are produced at rate \(s\) and die with a death-rate constant \(d\). Cells become infected with de novo infection-rate constant \(\beta\) and, once infected, die with rate constant \(c\). Hepatitis C virions are produced by infected cells at an average rate of \(p\) virions per cell per day and are cleared with a clearance rate constant \(c\). IFN in this model is assumed to reduce the production of virions from infected cells by a fraction \((1 - e)\) but could also reduce de novo infection of target cells by the factor \((1 - \eta)\). Before IFN therapy, \(\eta = \eta = 0\). IFN therapy is assumed to take effect, \(0 < e < 1\), at \(t = t_0\), where \(t = 0\) is the time of the first injection and \(t_0\) is a delay possibly because of a pharmacokinetic lag. On the assumption of a pretreatment steady state, the solution of equations (2) and (3), with \(T\) constant, is

\[V(t) = \begin{cases} V_0 \{A \exp[-\lambda_1(t - t_0)] + (1 - A) \exp[-\lambda_2(t - t_0)]\}, & t > t_0, \end{cases}\]

where

\[\lambda_{1,2} = \{(c + \delta + [(c - \delta)^2 + 4(1 - \eta)(1 - \eta)(c\delta)^2]^{1/2})\}/2;\]

\[A = (ec - \lambda_2)/(\lambda_1 - \lambda_2).\]

This solution was shown elsewhere [9] to give a biphasic decline similar to that of HCV decline under IFN treatment when the major effect of interferon is partially blocking virion production \((0 < e < 1)\). If an additional effect of blocking infection also occurs, the kinetics are not significantly changed. Here, as an approximation, we assume that \(\eta = 0\), but we have verified that the results presented here with regard to kinetic differences between genotype 1 and 2 are not affected if we assume that \(0 < \eta < 1\) (data not shown). Alternatively, an approximate solution of equation (3), where \(I(t)\) is assumed to be constant for a period of 2 days, is

\[V(t) \approx V_0 \{1 - e + e \exp[-c(t - t_0)]\}, \quad 2 > t > t_0\]

and can be used to fit the viral decline when only short-term data are available or as a first step in fitting the full set of data with equation (4) if there are enough data points during the first few days.

**Statistical analysis.** The Fisher’s exact test \((2 \times 2\) tables) and the \(\chi^2\) test \((N \times N\) tables) were used to determine the statistical significance of the distribution of categorical variables between groups. The nonparametric Wilcoxon rank sum test was used to
determine the statistical significance of differences in continuous variables among the different groups. Correlation among variables, or between variables and baseline values, was evaluated using the Spearman nonparametric test. Significance was established at $P < .03$.

**Nonlinear data fitting.** To estimate HCV viral kinetic parameters for each patient, the logarithm of equations (4) or (5) was fit to the logarithm of the virus load data by a nonlinear least-squares method using the DNLIS1 subroutine from the Common Los Alamos Software Library, which is based on a finite difference Levenberg-Marquardt algorithm [15]. Standard errors were calculated by the bootstrap method, in which experiments were simulated 100 times.

A mixed-effects approach [16] was also used for nonlinear fitting of the model to the virus-load data from days 0–2 or 0–14, of therapy. In this approach, which is the standard statistical method for the analysis of pharmacokinetic and other data from several subjects, each subject is assumed to have patient-specific dynamic parameters ($V_{ir}, I_0, c, d$, and $e$) normally distributed around mean values that may be different for each group of patients, depending on the HCV genotype or the IFN dose. In contrast to usual nonlinear regression analysis, where the parameters are estimated from each subject’s data separately and then summarized [9], in this approach data from all subjects are combined to estimate the population mean values directly while taking into account intersubject variation. This allows differences in mean values (e.g., because of HCV genotype) to be tested on the basis of all the data, in addition to taking into account possible differences due to dose and other factors. The logarithm of equations (4) or (5) was fitted to the logarithm of the virus load data on all evaluable subjects using a standard Newton-Raphson algorithm for nonlinear mixed-model analysis, implemented in the NLME function of the S-PLUS [17] statistical software package. Standard errors and test statistics for genotype and dose effects based on all subject data were calculated by the software via standard statistical theory.

**Results**

Table 1 lists the clinical demographics, histological findings, baseline alanine aminotransferase (ALT) level, and baseline virus load of the 8 patients infected with viral genotype 1 and the 9 patients infected with genotype 2. There were no differences between the two groups in the clinical and demographic features, histological activity, or baseline virus load (median, $9.7 \times 10^6$ cp/mL, with range $1.0 \times 10^4$–$5.9 \times 10^7$ for genotype 1 patients and median, $4.0 \times 10^5$, with range $4.0 \times 10^4$–$2.7 \times 10^7$ for genotype 2). Although the initial ALT was somewhat higher in patients infected with genotype 2 virus (median, 92; range, 54–242) than genotype 1 (median, 73; range, 31–157), this was not statistically significant.

After the initiation of IFN treatment, with 10 MU daily injections for 14 days, viral kinetics in both groups of patients followed a biphasic decline in most patients, with the exception of 2 patients who had a rebound after 1 day of therapy and 4 patients who interrupted treatment during the first week. The mean viral decline was faster and more profound in the genotype 2 patients, compared with genotype 1 patients (figure 1, top).

Nonlinear fitting (using equation [5]; see Methods) of the viral kinetic data was first performed for each individual patient for days 0–2 of treatment (figure 1, bottom), to obtain estimates of the initial virus load ($V_{ir}$), the delay in IFN effect ($t_{0}$), the effectiveness of IFN in blocking virus production ($e$), and the free virion clearance-rate constant ($c$). Results are given in table 2. The initial virus load and delay before virus levels began to fall were not significantly different between the genotypes; however, the mean effectiveness of IFN in blocking HCV production was significantly higher in genotype 2 patients than in genotype 1 patients ($e = 99.7 \% \pm 0.4 \%$ vs. $e = 95.3 \% \pm 4.0 \%$, $P = .003$; figure 2, top), which translates into a significantly larger mean viral decline at the end of 48 h for the genotype 2 group (2.95 log copies/mL), compared with the genotype 1 group (1.65 log copies/mL; figure 1, top). In addition, the free virion clearance rate ($c$) was significantly faster (figure 2, top) for patients with genotype 2 ($t_{1/2} = 2.0 \pm 0.5$ h) than for those with genotype 1 ($t_{1/2} = 3.0 \pm 1.0$ h; $P = .03$). No correlation was found between $e$ and $c$ for the genotype 2 patients alone or for the full set [$N = 23$] of genotype 1 patients alone, although both $e$ and $c$ are elevated for genotype 2, compared with genotype 1 patients.

Next, we fitted the viral kinetic data (using equation [4]; see Methods) during the first 14 days of treatment (figure 1, bottom), to estimate the death rate of infected cells ($d$), which is the major factor determining the second-phase slope (see results in table 2). The value of $d$ could not be estimated for patients who discontinued therapy early or whose virus load dropped below detectability by days 3–6 of treatment. The values estimated for $V_{ir}$, $t_{0}$, $e$, and $c$ from the fit of the data for days 0–2 were used to estimate $d$ when fitting the data for days 0–14.
The same parameter estimates, within the error boundaries, were found if equation (4) was used for estimating all 5 parameters at once using the 14-day data, but in some cases the nonlinear fitting algorithm failed to converge. Although the number of patients for whom \( \delta \), or the second-phase slope, can be estimated is small (\( N = 5 \) and 4 for genotypes 1 and 2, respectively), we nevertheless find that patients with genotype 2 have a significantly faster death rate of infected cells than do patients with genotype 1 (\( t_{1/2} = 3.2 \pm 1.7 \) days and \( t_{1/2} = 8.7 \pm 5.2 \) days, respectively; \( P = .03 \)). The infected-cell death rates for these 9 patients satisfy the inverse correlation with baseline virus load (\( R = -.8; P = .009; \text{figure 2, bottom} \)) that we observed elsewhere for 23 genotype 1 patients [9], but their limited number does not allow us to test this correlation independently for genotype 2.

To further verify that the differences between the genotypes are statistically significant, we have also used a nonlinear mixed-effects model [16] to analyze the complete genotype 1 data (23 patients [9] treated with 5, 10, or 15 MU of IFN), as well as the genotype 2 data presented here. The mean parameter values estimated for each genotype group (genotype 1: \( t_u = 7.7 \pm 0.7 \) h, \( \varepsilon = 97.0\% \pm 4.0\% \), \( c = 4.5 \pm 2.0 \text{ day}^{-1} \), \( \delta = .20 \pm .15 \text{ day}^{-1} \); genotype 2: \( t_u = 7.2 \pm 1.0 \) h, \( \varepsilon = 99.6\% \pm .7\% \), \( c = 8.6 \pm .9 \text{ day}^{-1} \), \( \delta = .36 \pm .25 \text{ day}^{-1} \)) are in agreement, within the standard deviations, with those obtained by fitting individual patients (table 2).

The mixed-effects analysis allows us to test in a multivariable approach the combined effect of both genotype and dose on each parameter in the model, taking into consideration random effects for each patient. We found that genotype is a very significant factor (\( P < .001 \)) for both the IFN effectiveness and free virion clearance rate and that it also influences the infected cell death rate but with a lower degree of significance (\( P = .02 \)). In addition, the mixed-effects model confirmed our result elsewhere [9] that an IFN dose of 5 MU gives significantly (\( P < .001 \)) lower blocking effectiveness than do doses of 10 or 15 MU (data not shown).

Finally, the fraction of patients that reached a virus level below detectability (<100 copies/mL) during the 14 days of induction therapy is significantly (\( P = .03 \)) higher in the genotype 2 group (4/5 patients who completed 14 days of treatment), compared with the genotype 1 group (1/7). In the genotype 2 group, 4/9 patients discontinued therapy on their own after 3–6 days of treatment, but only 1/8 genotype 1 patients receiving the same IFN dose discontinued therapy early. The patients who discontinued therapy had a strong early response, with a 2–4 log viral decline during the first 48 h of treatment and indications of a very rapid second-phase slope (e.g., patients 4C and 4E had the strongest responses in our study; figure 1, bottom).

**Discussion**

A number of controlled clinical studies have demonstrated that sustained virological response with either IFN monotherapy or IFN-ribavirin combination therapy is 2- to 3-fold greater in patients infected with HCV genotype 2, compared with patients infected with genotype 1 [5–8]. This holds true in IFN naïve patients as well as IFN-treated relapse patients [18]. Moreover, treatment of genotype 2 patients for 24 or 48 weeks results in the same long-term response rate, whereas for genotype 1–infected patients, the response rate increases with 48 weeks of treatment [5, 6]. The reasons for these differences in response with combination IFN and ribavirin therapy are not known and may involve both host and viral factors. Other host factors that have been associated with improved treatment response include age [6, 7], gender [6, 7], extent of hepatic damage [19], and race [20]. In this study, none of these factors differed significantly between the patient groups infected with genotype 1 and 2.
Table 2. Fitting results.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patient</th>
<th>Initial VL (log copies/mL)</th>
<th>Delay (h)</th>
<th>IFN-α effectiveness (%)</th>
<th>Virion clearance (c) Rate (1/day ± error)</th>
<th>Virion clearance (c) t½ (h)</th>
<th>Infected cell death (b) Rate (1/day ± error)</th>
<th>Infected cell death (b) t½ (days)</th>
<th>Production (10⁹ copies/day)</th>
<th>PCR at day 14 of therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1b</td>
<td>2A</td>
<td>6.8</td>
<td>7</td>
<td>86 ± 0.5</td>
<td>3.6 ± 0.2</td>
<td>4.6</td>
<td>0.12 ± 0.01</td>
<td>5.8</td>
<td>410</td>
<td>Positive</td>
</tr>
<tr>
<td>1b</td>
<td>2B</td>
<td>7.2</td>
<td>9</td>
<td>98 ± 0.0</td>
<td>6.0 ± 0.3</td>
<td>2.8</td>
<td>0.11 ± 0.03</td>
<td>6.3</td>
<td>1089</td>
<td>Positive</td>
</tr>
<tr>
<td>1a</td>
<td>2C</td>
<td>6.9</td>
<td>8</td>
<td>96 ± 1.0</td>
<td>6.8 ± 0.76</td>
<td>2.5</td>
<td>0.16 ± 0.04</td>
<td>4.3</td>
<td>92</td>
<td>Positive</td>
</tr>
<tr>
<td>1a</td>
<td>2D</td>
<td>6.0</td>
<td>7</td>
<td>95 ± 1.0</td>
<td>5.6 ± 0.5</td>
<td>3.0</td>
<td>0.07 ± 0.02</td>
<td>9.9</td>
<td>12,191</td>
<td>Positive</td>
</tr>
<tr>
<td>1a</td>
<td>2E</td>
<td>7.8</td>
<td>10</td>
<td>99.7 ± 0.01</td>
<td>11.2 ± 0.6</td>
<td>1.5</td>
<td>0.04 ± 0.01</td>
<td>17.3</td>
<td>965</td>
<td>NA</td>
</tr>
<tr>
<td>1b</td>
<td>2F</td>
<td>7.0</td>
<td>7</td>
<td>96 ± 0.9</td>
<td>4.4 ± 0.07</td>
<td>3.8</td>
<td>0.04 ± 0.01</td>
<td>17.5</td>
<td>965</td>
<td>NA</td>
</tr>
<tr>
<td>1b</td>
<td>2G</td>
<td>7.4</td>
<td>7</td>
<td>92 ± 0.8</td>
<td>4.8 ± 0.1</td>
<td>3.5</td>
<td>RB</td>
<td>1780</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>2H</td>
<td>6.4</td>
<td>9</td>
<td>99.3 ± 0.2</td>
<td>7.9 ± 1.0</td>
<td>2.1</td>
<td>ND</td>
<td>324</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.10 ± 0.05</td>
<td>8.7 ± 5.2</td>
<td>2282 ± 4045</td>
<td>1/7 Negative</td>
</tr>
<tr>
<td>2b</td>
<td>4A</td>
<td>6.6</td>
<td>5</td>
<td>99.3 ± 0.4</td>
<td>5.4 ± 0.3</td>
<td>3.1</td>
<td>0.12 ± 0.04</td>
<td>5.8</td>
<td>417</td>
<td>Positive</td>
</tr>
<tr>
<td>2b</td>
<td>4B</td>
<td>6.5</td>
<td>9</td>
<td>99.1 ± 0.5</td>
<td>9.2 ± 3.7</td>
<td>1.8</td>
<td>NA</td>
<td>497</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>2b</td>
<td>4C</td>
<td>6.8</td>
<td>5</td>
<td>99.99 ± 0.5</td>
<td>8.8 ± 0.7</td>
<td>1.9</td>
<td>NA</td>
<td>955</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2b</td>
<td>4D</td>
<td>6.6</td>
<td>8</td>
<td>98.91 ± 0.2</td>
<td>7.6 ± 0.8</td>
<td>2.2</td>
<td>0.3 ± 0.05</td>
<td>2.3</td>
<td>446</td>
<td>Negative</td>
</tr>
<tr>
<td>2b</td>
<td>4E</td>
<td>7.4</td>
<td>6</td>
<td>99.99 ± 0.4</td>
<td>10.4 ± 0.9</td>
<td>1.6</td>
<td>NA</td>
<td>4248</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2b</td>
<td>4G</td>
<td>6.4</td>
<td>7</td>
<td>99.94 ± 0.3</td>
<td>8.7 ± 0.6</td>
<td>1.9</td>
<td>NA</td>
<td>359</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2b</td>
<td>4H</td>
<td>6.6</td>
<td>10</td>
<td>99.8 ± 0.3</td>
<td>12.4 ± 0.6</td>
<td>1.3</td>
<td>0.27 ± 0.03</td>
<td>2.6</td>
<td>801</td>
<td>Negative</td>
</tr>
<tr>
<td>2a</td>
<td>4I</td>
<td>6.6</td>
<td>9</td>
<td>99.93 ± 0.3</td>
<td>9.0 ± 0.6</td>
<td>1.8</td>
<td>0.34 ± 0.03</td>
<td>2.0</td>
<td>732</td>
<td>Negative</td>
</tr>
<tr>
<td>2b</td>
<td>4J</td>
<td>5.6</td>
<td>6</td>
<td>99.93 ± 0.2</td>
<td>7.6 ± 0.5</td>
<td>2.2</td>
<td>ND</td>
<td>46</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.26 ± 0.10</td>
<td>2.3 ± 0.3</td>
<td>845 ± 1268</td>
<td>4/5 Negative</td>
</tr>
</tbody>
</table>

NOTE. VL, virus load; IFN-α, interferon-α; NA, patient discontinued treatment before day 14; RB, rebounder (VL decreased ≥1 log but rebounded after 1 day by ≥0.5 log); ND, VL below sensitivity (100 copies/mL) from day 3 of treatment.

a Difference between genotype 1 and genotype 2 is significant at P < 0.01.
b Difference between genotype 1 and genotype 2 is significant at P < 0.05.

Here we have examined whether the early viral kinetics in response to IFN treatment differed between patients infected with genotypes 1 and 2. Both groups responded in a biphasic fashion, with an initial rapid decline in serum HCV followed by a slower second-phase decline. In the genotype 2-infected patients, however, the extent of decline during the first phase was significantly greater than that observed for the genotype 1 patients. This difference can be explained in part by a greater effectiveness of IFN in blocking the production of HCV virions in genotype 2 infection. In addition, a faster rate of decline during the first phase in genotype 2 patients also reflected a higher free virion clearance rate. Finally, the decline slope during the second phase was also significantly faster in genotype 2–infected patients, although the number of patients for whom we could assess this slope was small. Similar analysis in another study, with a larger number of patients but less frequent sampling, also shows a significantly faster second-phase slope in genotype 2 patients (A. Neumann, R. Reddy, T. Layden, and J. Poulakos, unpublished data). This difference in the slope of second-phase decline has been attributed to differences in the possibly immune-mediated death rate of HCV-infected cells [9].

What are the possible mechanisms underlying the observed differences in kinetics? The response to IFN in genotype 1b infection has been shown in some studies [21–23] to correlate either with the number of amino acid changes in codons 2209–2248, the interferon sensitivity determining region (ISDR) of the nonstructural protein 5A (NS5A) or with specific mutations in the ISDR. Also, it was recently shown that, in patients infected with HCV genotype 2a, a large number of mutations in the corresponding ISDR (codons 2163–2228) is associated with higher response rate to IFN treatment [24]. The proposed mechanism for this correlation relates to studies demonstrating that the NS5A protein from the ISDR of wild-type genotype 1a and 1b virus can complex with RNA-dependent protein kinase (PKR) and diminish its ability to inhibit viral protein translation [25, 26]. A large number of amino acid changes in the wild-type ISDR make HCV more sensitive to IFN. Thus, if the genotype 2 ISDR has more mutations, or if its mutations give rise to less resistance to IFN than those in genotype 1, then the differences between the genotypes that we observe in n could be explained. There are, however, a number of studies demonstrating no correlation between IFN response and NS5A structure in genotype 1 virus [27–29]. Thus, the relation between mutations in the ISDR, genotype, and the IFN effectiveness in blocking production requires further study.

Another possible explanation for the differences in the IFN blocking effectiveness between the genotypes is related to the HCV second envelope glycoprotein (E2), which has significant sequence variations between virus strains. E2 has a relatively conserved 12–amino acid sequence from most virus isolates that is similar to the PKR phosphorylation site and the translation initiation factor eIF2α phosphorylation site [30]. It was found [30] that this 12–amino acid sequence in genotype 1a and 1b virus more closely resembles the PKR-eIF2α homology domain than the less IFN-resistant virus strains, genotypes 2 and 3. The authors demonstrated that the genotype 1a and 1b E2 proteins were capable of inhibiting PKR activity in vitro and its effect on cellular function and growth. Mutations in the E2 sequence, simulating the E2 sequence in genotype 2, prevented this inhibition [30]. Our results, demonstrating a significantly
Figure 2. Differences between kinetic parameters in patients infected with hepatitis C virus (HCV) genotype 1 (●) and genotype 2 (○). Top, Effectiveness of interferon (IFN)-α in blocking virus production (e) and the free virion clearance rate constant (c). Bottom, Death rate of infected cells (d) plotted against the baseline virus load ($V_0$).

greater degree of interferon effectiveness in blocking virion production in genotype 2 versus genotype 1 virus, strongly support the suggestion that HCV of genotype 1 is more capable in counteracting the effects of IFN on viral translation. Both of the genetic differences between the genotypes, in NS5A and in E2, could lead to this diminished IFN effectiveness.

The faster clearance of HCV genotype 2 virus during the first 48 h of treatment (first phase) was due not just to a difference in IFN effectiveness but also a faster free virion clearance rate. This clearance rate, which reflects the half-life of virions in the serum, may be enhanced by antibody-mediated viral clearance in addition to the intrinsic nonspecific clearance of virions in the body. Indeed, a number of studies [31–33] have shown that antibody responses to the hypervariable region 1 (HVR-1) of the HCV envelope glycoprotein E2 were significantly more vigorous and frequent in HCV genotype 2 infected subjects, compared with genotype 1 patients. Furthermore, the more rapid variation in HVR-1 viral sequences from genotype 2 patients, in comparison with genotype 1 [34], also suggests a stronger antibody immune pressure on genotype 2 virus.

Finally, the second phase of viral decline was significantly faster in genotype 2–infected patients, compared to genotype 1. This slope of viral decline has been shown to vary widely in patients infected with genotype 1 virus and is the best predictor of early viral clearance [9]. The faster second phase viral decline most likely reflects a greater degree of immune mediated recognition and killing of HCV-infected cells. Indeed, it was found [35] that the specific anti-HCV proliferative response of CD4 cells was significantly stronger, and more enhanced by IFN therapy, in patients infected with genotype 2 as compared with genotype 1 patients. Whether a greater killing of genotype 2–infected cells occurs will require a more careful comparison between the CTL response in genotype 2– and genotype 1–infected patients.

Another hypothesis for the better response to treatment in patients infected with HCV genotype 2 is that the replication of this genotype is slower than that of genotype 1. From the results presented here, however, there is no evidence for this. In our study, virion production (estimated here by baseline virus load multiplied by the virion clearance rate) is not significantly faster or slower in genotype 2 patients, because we find that genotype 2 patients have faster clearance rates but somewhat reduced baseline virus loads. Nevertheless, in a study [36] analyzing differences in baseline virus load between HCV genotypes with a large number of patients, it was found that genotype 2 patients have significantly lower virus loads than do genotype 1 patients. A large study with frequent kinetics of genotype 2 HCV during treatment or after its cessation is needed to determine possible difference in replication rates between the genotypes. It is also interestingly to note that HCV of genotype 2 has been found to have significantly lower levels of both minus-strand and genomic-strand RNA in the liver [37]. In that study, however, the patients infected with genotype 2 had plasma baseline virus loads comparable to those of the genotype 1 patients independent of virus load in liver. Thus again, the question of differences in replication rates between the genotypes cannot be answered.

The current results provide evidence that the better response to IFN in genotype 2–infected patients is multifactorial, reflecting differences in IFN’s capability of inhibiting virus production and differences in virion clearance and removal of infected cells, possibly reflecting differences in both the humoral and cellular immune response to the virus. This is the first finding of a difference in viral dynamics between subtypes of the same virus and shows the importance of subtype-specific interactions between the virus, the host, and the drug used for treatment. Of clinical importance is our finding that the predictive value of HCV genotype for the success of treatment [38] may correlate with differences in early viral kinetics observed between groups of patients infected with different genotypes.
Although faster decay of genotype 2 virus characterizes the mean response in that group, it is not observed for all genotype 2 patients. On the other hand, faster viral decay early in treatment correlates with viral negativity at 12 weeks [9] and with end of treatment and sustained virological responses [39]. Thus, the analysis of early viral kinetics may be essential, alone or together with baseline virus load and HCV genotype, for making better predictions of response to therapy.

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References
