Number and Position of Mutations in the Interferon (IFN) Sensitivity–Determining Region of the Gene for Nonstructural Protein 5A Correlate with IFN Efficacy in Hepatitis C Virus Genotype 1b Infection

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To explore the relationship between responses to interferon (IFN) and the mutation patterns in the IFN sensitivity–determining region (ISDR; amino acid positions 2209–2248) in the NS5A gene of hepatitis C virus genotype 1b, a cohort of 334 patients was analyzed. The number of mutations in the ISDR was higher in patients with sustained response (SR) than in patients with transient or no response (P < .001). Patients with viruses mutated at positions 2209 (P = .02), 2216 (P = .01), or 2227 (P = .02) more frequently experienced SR than did those without these mutations. Mutation occurred most frequently at position 2218, where the presence of cysteine was significantly associated with SR. Thus, the mutation pattern in the ISDR affects the virologic response to IFN and reflects different influences on the function of the NS5A protein. ISDR sequence analysis would allow the prediction of clinical IFN efficacy in individual patients.

We previously reported a close correlation between the number of mutations in the NS5A gene encoding amino acids (aa) at positions 2209–2248 (the interferon [IFN] sensitivity–determining region [ISDR]) and IFN efficacy in patients with hepatitis C virus (HCV) genotype 1b infection [1–3]. Most patients with ≥4 mutations in the ISDR (thereby classified as mutant type) experienced sustained responses (SRs), which are defined as a patient’s testing negative for serum HCV RNA for 6 months after the end of IFN treatment. Other investigators confirmed this correlation, but mainly in Japan [4–6]. In Western countries, results have been conflicting [7–12], and only some investigators reported data similar to those of the Japanese studies [13, 14]. Moreover, Sarrazin et al. [15] reported a correlation between the number of mutations and IFN efficacy in patients with HCV-1b treated with combination therapy (IFN and ribavirin). Therefore, it is important to clarify definitively the relationship between ISDR mutations and the clinical efficacy of IFN in a large number of patients.

There are diverse sequence patterns in the ISDR, but the clinical and virologic significance of each mutation has yet to be studied in detail. Mutations at different positions in the ISDR and involving different mutations appear to affect IFN sensitivity differently—even among persons with the same number of ISDR mutations. This suggests that the IFN sensitivity of each person could be related not only to the number of mutations but also to the type of mutation pattern in the ISDR.

The prognosis for patients with transient virologic responses (TRs), defined as a patient’s testing negative for serum HCV RNA by the end of IFN treatment but testing positive for serum HCV RNA thereafter, differs from that for patients with a “nonresponse” (NR), defined as a patient’s remaining positive for HCV RNA at the end of IFN treatment. Response rates to retreatment with IFN monotherapy [16] or combination therapies with ribavirin and IFN [17] differ, as does normalization of alanine aminotransferase (ALT) levels, after the end of IFN treatment (biochemical responder), which leads to a lower rate of subsequent hepatocellular carcinoma (HCC) development [18, 19]. Because virologic factors to predict TR and NR have not been fully elucidated, it is important to study the influence of ISDR sequences on different virologic responses to IFN. Thus, we investigated the influence of ISDR mutations on clinical IFN efficacy for 334 patients and analyzed the relationship between the ISDR sequence pattern and virologic responses to IFN.

Patients and Methods

Patients. We retrospectively analyzed 334 patients with chronic HCV-1b infection who received IFN therapy between 1994 and 1998 at Tokyo Medical and Dental University Hospital and its
affiliated hospitals. Of these patients, 83 were included in our previous study [2]. All had chronic, active hepatitis infection, with positive anti-HCV antibodies and serum HCV RNA, as determined by reverse transcription (RT)–polymerase chain reaction (PCR) assay with the HCV Amplicor kit (Roche Diagnostic Systems; detection limit, 100 copies of viral genome/mL of serum) [20].

All 334 patients were infected with HCV-1b, as determined by a mixed primer PCR assay targeted to the core region of the HCV genome [21], which can efficiently discriminate 3 genotypes (HCV-1b, -2a, and -2b) that compose >99% of Japanese HCV infection [22]. All patients tested negative for serum hepatitis B surface antigen and antinuclear antibodies and had no other causes of hepatitis, including excessive alcohol consumption. Those with negative serum HCV RNA for 6 months after the end of IFN treatment were defined as having SR. Patients in whom HCV RNA became negative at the end of IFN treatment but reappeared during the follow-up period (≤6 months) were considered to have TR. Patients who did not become negative for HCV RNA by the end of treatment were considered to have NR.

All patients received intramuscular IFN-α or intravenous IFN-β with a dose of 6–10 × 10^6 U 3 times a week for 6 months, with initial daily administration for 2–8 weeks (total dose, 480–1060 × 10^6 U). This schedule is a standard regimen in Japan. The regimen followed the guidelines for IFN therapy of chronic hepatitis C approved by a governmental authority (National Health Insurance of Japan) and was in accordance with the Helsinki Declaration of 1975, as revised in 1983. Patients were monitored monthly with serial determinations of ALT levels. Serum was tested for HCV RNA just before therapy and then every 3 months by Amplicor HCV kit.

We analyzed the following factors to determine whether they were related to the efficacy of IFN: age, sex, stage of fibrosis at liver biopsy, total IFN dose, pretreatment serum ALT level, serum HCV RNA level (virus titer), and aa sequence of the ISDR before treatment. Liver biopsy specimens were evaluated by an independent interpreter, who was not aware of the patient’s infection status, and were ranked according to the stage of fibrosis (mild, moderate, or severe) [23]. Serum HCV RNA levels were determined by a branched-chain DNA assay (Quantiplex HCV RNA version 1.0; Chiron) [24]. The limit of detection of this assay was 0.5 × 10^5 genome equivalents/mL.

Sequence analysis of the ISDR. We extracted RNA from serum by using a modified acid guanidinium thiocyanate–phenol-chloroform extraction (AGPC) method [25] with ISOGEN (Wako). RT-PCR analysis was done, as described elsewhere [26], with a hot start PCR technique with the AmpliWax PCR Gem 50 (Takara). In brief, RNA extracted from 150 μL of serum was reverse transcribed to cDNA by using random hexamers. This cDNA was used for the first PCR assay. Fifty microliters of the lower mixture consisted of 1.5 mM MgCl₂, 400 nM each PCR primer (outer primer), and 200 nM dNTP. This was separated by a wax layer from 50 μL of the upper PCR mixture containing 1 μL of the cDNA solution, 10 mM Tris-HCl (pH 8.3), 0.01% gelatin, and 0.5 U of Taq polymerase (AmpliTaq; Promega) until PCR began and the wax layer melted. The PCR assay was done according to the following schedule: 40 cycles of denaturing at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min followed by extension at 72°C for 7 min. We transferred 1 μL of the first PCR product to the upper mixture of the second PCR assay. Other conditions for the second PCR assay were the same as for the first PCR assay, except that we used inner primers instead of outer primers.

To determine the nucleotide (nt) sequence of NS5A 2209–2248, we amplified nt 7296–7320 of HCV cDNA by using the outer set of primers (5′ outer primer, 5′-TGGATGGAGTGGGTTGACAG GTA-3′ [nt 6703–6727 of HC-J4]; 3′ outer primer, 5′-TCTTTTCTCCTGGGAGGTGTATTGC-3′ [nt 7296–7320]). We transferred 1 μL of the first PCR product to the second PCR reaction along with the nested 5′ and 3′ primers (5′ inner primer, 5′-TGGAAAGCAGCGCCAGTCA GTGATCGCTTCTCCGCTGCA-3′ [nt 6772–6741], with the M13 forward primer sequence underlined; and 3′ inner primer, 5′-CAGGAAACAGCTATGACCCGGGCTTGTAGTGGCAA-3′ [nt 7275–7294], with the M13 reverse primer sequence underlined). An M13 forward primer and an M13 reverse primer were attached to the 5′ terminal of the 5′ and 3′ inner primers, respectively, to facilitate direct sequencing with an automated DNA sequencer (model 373S; Applied Biosystems Japan).

Both strands of the PCR products were sequenced with the PRISM dye termination kit (Applied Biosystems Japan), according to the manufacturer’s instructions. The sequencing primer was the M13 forward primer for the sense strand and the M13 reverse primer for the antisense strand. Deduced aa sequences of NS5A 2209–2248 were compared with the NS5A 2209–2248 sequences of HCV-J [27], which are prototypic sequences of HCV-1b. We defined HCV with no mutations in the ISDR as wild type, HCV with 1–3 mutations in the ISDR as intermediate type, and HCV with >4 mutations in the ISDR as mutant type, compared with HCV-J, as described elsewhere [2]. In addition, we also analyzed NS5A aa sequences 2249–2274, which consist of the carboxy terminal half of double-stranded RNA-activated protein kinase (PKR) binding domain (NS5A 2209–2274) [28], in the subgroup of 146 randomly selected patients. We defined different aa, compared with the consensus sequence (KVVILSDFAEDEREVSSVAEI, standard single letter aa codes) of these 146 patients, as mutations of this region. The results of the sequencing analysis were confirmed as consistent for each sample by repeating the experiment twice with different PCR products, to rule out the possibility of selection and amplification of minor NS5A quasi species variants in the low-titer specimens.

Statistical analysis. We compared categorical data by the χ² or Fisher’s exact test. Distributions of continuous variables were analyzed by the Mann-Whitney U or Student’s t test for 2 groups and by the Kruskal-Wallis test or analysis of variance with adjustment for multiple comparisons by the Scheffe method for 3 groups, as appropriate, with Statview 5.0 software (Abacus Concepts). A correlation between the number of mutations in the ISDR and serum HCV RNA levels was analyzed by Spearman’s rank correlation test. Multivariate analysis was done by multiple logistic regression analysis (JMP program; SAS Institute). All tests of significance were 2-tailed. P < .05 was considered to be statistically significant.

Results

Figure 1 delineates the sequence variations found in the ISDR in viruses from the entire cohort of 334 patients and also summarizes the patients’ IFN responses. In all, 145 patients
Figure 1. Interferon (IFN) sensitivity–determining region (ISDR) sequence and IFN response in 334 patients. Amino acid residues are indicated by standard single-letter codes; dashes indicate residues identical to those of hepatitis C virus–J sequence (shown at top). ISDR wild type represented 44% (145 of 334) of all patients, intermediate type represented 40% (135 of 334), and mutant type represented 16% (54 of 334). In all, 54 mutant-type sequences were extremely diverse, with each patient having a unique mutant sequence. As summarized in table 1, in patients with wild and intermediate types, sustained response (SR) occurred in 8% (12 of 145) and 15% (20 of 135), respectively, vs. 91% (49 of 54) of patients with mutant type. NR, nonresponse; TR, transient response.

Table 1. Relationships between interferon (IFN) sensitivity–determining region (ISDR) types and IFN efficacy in 334 patients with chronic hepatitis C virus infection.

<table>
<thead>
<tr>
<th>Response</th>
<th>Wild (n = 145)</th>
<th>Intermediate (n = 135)</th>
<th>Mutant (n = 54)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonresponse</td>
<td>55 (38)</td>
<td>49 (36)</td>
<td>0</td>
<td>.001</td>
</tr>
<tr>
<td>Transient</td>
<td>78 (54)</td>
<td>66 (49)</td>
<td>5 (9)</td>
<td>.001</td>
</tr>
<tr>
<td>Sustained</td>
<td>12 (8)</td>
<td>20 (15)</td>
<td>49 (91)</td>
<td>.001</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of patients.

* Mutant vs. wild type and mutant vs. intermediate type (χ² test).

(44%) had wild type, 135 (40%) had intermediate type, and 54 (16%) had mutant type viral ISDR. Fifty-four mutant type sequences were extremely diverse, with each patient having a unique mutant sequence.

Among these 334 patients, 104 (31%) had NR, 149 (45%) had TR, and 81 (24%) had SR (table 1). In 145 patients with wild-type viral ISDR, 55 (38%) had NR, 78 (54%) had TR, and 12 (8%) had SR; and among 135 patients with intermediate-type viral ISDR, 49 (36%) had NR, 66 (49%) had TR, and 20 (15%) had SR. No patient with mutant-type viral ISDR failed to respond to IFN; 5 (9%) had TR, and 49 (91%) had SR. The SR rate was significantly higher, whereas NR and TR rates were significantly lower in mutant type, compared with wild- or intermediate-type ISDRs. However, there were no significant differences in the responses of patients with wild- and intermediate-type viral ISDR.

There were significant differences in sex (P = .004), ALT level (P = .002), virus titer (P < .001), and number of ISDR mutations (P < .001) between SR and NR patients and in ALT level (P = .022), virus titer (P < .001), number of mutations in ISDR (P < .001), and number of mutations in the carboxy terminal half.
of the PKR-binding domain (aa 2249–2274, \( P = .037 \)) between SR and TR patients by univariate analysis (table 2). Serum ALT level was marginally higher in TR than in NR patients (\( P = .047 \)). Subsequently, we analyzed these clinical backgrounds by multivariate analysis among 3 groups, to identify independent predictors for SR in all patients and for TR in TR and NR patients. The number of ISDR mutations (odds ratio [OR], 2.123; \( P < .001 \)), virus titer (OR, 0.628; \( P < .001 \)), and ALT level (OR, 1.004; \( P = .013 \)) were independently associated with SR. However, no clinical factors were independently associated with TR, compared with NR patients (table 3).

The SR rates were 8\% (12 of 145), 13\% (13 of 102), 17\% (4 of 23), 30\% (3 of 10), 78\% (7 of 9), and 93\% (42 of 45) among patients who had 0, 1, 2, 3, 4, or \( \geq 5 \) mutations, respectively, in the viral ISDR, which shows that the SR rate was correlated with the number of mutations in the ISDR (\( P < .001 \), Spearman’s rank correlation test; figure 2). All patients with mutant-type HCV experienced SR or TR; thus, patients with mutant-type ISDR have a significant tendency to SR or TR, compared with wild and intermediate types (\( P < .001 \), \( \chi^2 \) test). In patients with \( \leq 3 \) mutations in the ISDR (wild type or intermediate type), we found no significant differences in the ratio of NR (40\%–54\%) versus TR (30\%–38\%) associated with the number of mutations in the ISDR.

Figure 3 documents a correlation between the number of ISDR mutations and HCV titer. Virus titers were inversely correlated with the number of aa mutations in the ISDR (\( P < .001 \); \( r = -.372 \), Spearman’s rank correlation test), as expected from an association between the clinical IFN response, HCV titer, and ISDR mutations by univariate analysis, as shown in table 2.

Subsequently, we analyzed the prevalence of mutations at each different position in the ISDR and compared patients with or without SR (figure 4). Among the 40 aa in the ISDR, mutations were most frequent at position 2218 (127 [38\%] of 334), where arginine was most frequently found in place of the wild-type histidine. When all patients were analyzed, mutations at any of the 22 residues shown in figure 4 were more frequent in SR patients (Fisher’s exact probability test). However, the influence of individual mutations on the clinical response to IFN should not be overestimated, because most mutant-type patients had sustained responses, regardless of the ISDR mutation site. Therefore, we analyzed this relationship only in patients with intermediate-type viral ISDR mutation, to define the specific influence of a mutation site while minimizing the influence of mutation number in the ISDR (figure 4B). In patients categorized as being limited to intermediate type, mutations at positions 2209 (\( P = .02 \)), 2216 (\( P = .01 \)), or 2227 (\( P = .02 \)) were associated with a significantly higher response rate than in the absence of these mutations (Fisher’s exact probability test). Although the prevalence of mutations at positions 2218 and 2224 was high in patients with SR (70\% [14 of 20] and 15\% [3 of 20], respectively), this was also the case in patients without SR (66\% [76 of 115] and 22\% [25 of 115], respectively), resulting in no significant association between these mutations and SR.

Finally, we analyzed the relationship between the SR rate and the type of aa present at position 2218, where mutations were most frequent of all 40 aa of the ISDR (table 4). The SR rate was 8\% (12 of 145) for wild type (where position 2218 is histidine) and 13\% (8 of 61), 38\% (3 of 8), and 0 (0 of 6) with 1 mutation at 2218 to arginine, cysteine, and asparagine, respectively. In patients with a single mutation at position 2218, the SR rate was significantly higher when cysteine was present, compared with any other aa (\( P = .03 \), Fisher’s exact probability test). Similarly, in patients with 1–3 mutations in the viral ISDR (excluding position 2218), a higher SR rate was found with cysteine and asparagine at position 2218 than with histidine or arginine. In all 334 patients, the SR rate was significantly higher in persons with cysteine (65\% [9 of 14]) than with histidine.
Table 3. Multivariate analysis for the clinical and virologic factors related to sustained response (SR) to interferon (IFN) in 334 patients with chronic hepatitis C virus infection and to transient response (TR) in non-SR patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with SR</th>
<th>Patients with TR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Sex, male vs. female</td>
<td>0.69 (0.29–1.66)</td>
<td>.410</td>
</tr>
<tr>
<td>Age, per year</td>
<td>0.98 (0.95–1.01)</td>
<td>.247</td>
</tr>
<tr>
<td>ALT, per U/L</td>
<td>1.004 (1.001–1.007)</td>
<td>.013</td>
</tr>
<tr>
<td>IFN dose, per million U</td>
<td>0.999 (0.997–1.002)</td>
<td>.715</td>
</tr>
<tr>
<td>Fibrosis stage, F1/2/3</td>
<td>0.57 (0.31–1.05)</td>
<td>.071</td>
</tr>
<tr>
<td>Virus titer, per Meq/mL</td>
<td>0.63 (0.51–0.77)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>No. of mutations in ISDR</td>
<td>2.12 (1.68–2.68)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>No. of mutations in NS5A 2249–2274</td>
<td>1.69 (0.75–3.59)</td>
<td>.639</td>
</tr>
</tbody>
</table>

NOTE. ALT, alanine aminotransferase; CI, confidence interval; ISDR, IFN sensitivity–determining region.

*Multivariate analysis was done in subcohort of 146 randomly selected patients with ALT, virus titer, no. of mutations in ISDR, and no. of mutations in carboxy half of RNA-activated protein kinase–binding domain (NS5A 22492–274).

Discussion

In this study, we analyzed the relationship between ISDR aa mutation patterns and virologic response to IFN in 334 patients with chronic HCV-1b (figure 1). There were 4 main findings: first, there were significantly more mutations in the ISDR in patients with SR than in those with TR or NR but no significant difference between those with TR and NR; second, the SR rate correlates with the number of mutations in the ISDR; third, mutations at positions 2209, 2216, and 2227 are significantly associated with SR; and fourth, a cysteine residue at position 2218 is associated with SR. Therefore, both the type and number of mutations in the ISDR affects the virologic response to IFN in chronic HCV-1b infection. This may be explained by proposing that different mutations in the ISDR have different effects on the function of the NS5A protein.

Persons with more mutations in the ISDR had higher SR rates, even within the same category of ISDR type (i.e., intermediate or mutant types; figure 2). Small differences in the number of ISDR mutations could profoundly affect the IFN sensitivity of HCV. For example, patients with 5 mutations experienced higher response rates than those with 4 mutations. In addition, the number of mutations in the ISDR was inversely correlated with serum HCV RNA levels (figure 3), and persons with more mutations in the ISDR had lower serum HCV RNA titers. Although these findings extend our previous results of associations among ISDR and SR rates and virus load, the mechanism for this interrelationship has yet to be clarified. In one possible explanation, the NS5A protein is needed for efficient viral replication, and the ISDR mutation impairs this function, which results in decreased virus load. In this case, increased susceptibility to IFN is secondary to inefficient replication of HCV. Otherwise, the NS5A protein itself may have the anti-IFN function, such as PKR repression [29] or inhibition of IFN signal transduction [30], and decreased virus load in the ISDR mutant type is caused by the effect of the endogenous IFN. It seems important to elucidate these points in further research.

We found that not only the number but also the pattern of mutations in the ISDR affected IFN sensitivity (figure 4). When we analyzed the mutation pattern in all patients, 22 of 40 aa positions in the ISDR were significantly related to IFN sensitivity. Because of the high SR rate in the mutant type, most mutation sites observed in that type appeared to affect IFN sensitivity. However, in the intermediate type, we could analyze the effect of each mutation on IFN sensitivity separately because of the
fewer mutations and the lower SR rate in this type. This analysis revealed that, even in HCV with a similar number of ISDR mutations, HCV with mutations at positions 2209, 2216, or 2227 was more sensitive to IFN. This suggests that these residues have a critical effect on NS5A protein function. Although the role of each aa residue in ISDR function is largely unknown, results in this study indicate that not only the ISDR mutation number but also the mutation site affects IFN efficacy. Furthermore, the SR rate is significantly higher in patients with HCV in which position 2218 was C, rather than any other residue. This suggests that cysteine mutation at position 2218 changes NS5A function more profoundly than other aa. Such information is valuable in clinical settings for predicting IFN efficacy in individual patients with a small number of mutations.

Studies of the biologic functions of NS5A have revealed some aspects of its properties. Gale et al. [28, 29, 31, 32] showed that the NS5A protein inhibits an RNA-dependent PKR. The NS5A protein forms a complex with the PKR through the ISDR and inhibits the phosphorylation of eukaryotic translation initiation factor–2α, a repressor of viral protein translation initiation, and mutations in the ISDR block this interaction. These studies identified the PKR-binding domain consisting of ISDR (aa 2209–2248) and its downstream 26 aa of NS5A (aa 2249–2274); a European study showed that mutations in the downstream region of the PKR-binding domain were more closely associated with IFN efficacy than the ISDR itself in HCV-1a infection [33]. In the present study, although the mutations in the downstream region were related to the IFN effect similarly to those of the ISDR, multivariate analysis showed that the ISDR mutations were more directly related to IFN effect than to the carboxy-terminal region of the PKR-binding domain, which probably reflects the functional significance of these 2 regions, especially in HCV-1b infection.

Many reports discuss the mechanism of IFN resistance of HCV other than PKR inhibition. Francois et al. [34] showed that expression of all HCV proteins allowed the development of a partial resistance to the antiviral action of IFN in the cultured cells. However, the resistance of these cells to IFN was not attributed to inhibition of PKR, which raises the possibility that some HCV proteins may have other functions to repress IFN action. Hein et al. [30] found that expression of HCV proteins in cell lines strongly inhibited IFN-α–induced signal transduction through the Jak-STAT pathway. It was not clear which HCV protein inhibited IFN-α–induced signal transduction, but NS5A may have this function, since ISDR sequences are closely related to IFN effect, as shown in the present study. Further studies about the influence of the NS5A protein on the Jak-STAT pathway are necessary. Nousbaum et al. [35] recently reported that the selection of variable stretch 3 (V3) region (aa 2356–2379) quasi species was observed within the first 2–6 weeks of IFN plus ribavirin therapy in responders, but not in nonresponders, with genotype 1a and 1b. This indicates that both the ISDR and the carboxy terminal of NS5A may be correlated with IFN sensitivity. Other investigations revealed...
that the HCV-1b NS5A protein has a transcriptional activator function [36, 37], but the significance of this activity on IFN sensitivity is not known. Thus, the mechanism by which IFN eradicates persistent HCV infection remains unclear, and the functional significance of the aa mutations seen in the present study needs more investigation.

We did not find any virologic differences between TR and NR patients, including differences in ISDR types. This indicates that ISDR mutations exclusively associate with SR and do not discriminate between TR and NR. Transient viral responses to IFN during treatment followed by relapse are nevertheless reported to be associated with a more favorable prognosis of chronic hepatitis, such as normalization of ALT level after the end of IFN treatment (biochemical responder), which leads to a lower rate of subsequent HCC development [18, 19], or with higher response rates to combination therapy with IFN plus ribavirin [38, 39]. This suggests that TR and NR are different clinical and virologic entities, despite persistent viremia after IFN therapy in both groups. Therefore, it would be useful to elucidate the virologic difference between these 2 groups for better understanding of HCV pathogenesis or therapeutic strategy. We previously reported an association between the number of mutations in the ISDR and the SR but did not study whether such an association existed in TR, prompting us to analyze here whether mutations in the ISDR could distinguish TR from NR. Among wild- and intermediate-type ISDR, the ratio between

Table 4. Rate of sustained response to interferon (IFN) therapy in patients infected with hepatitis C virus and possessing different amino acid (aa) residues at position 2218.

<table>
<thead>
<tr>
<th>No. of mutations in ISDR (except position 2218)</th>
<th>aa Residue at position 2218</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Histidine</td>
<td>Arginine</td>
</tr>
<tr>
<td>0</td>
<td>8 (12/145)</td>
<td>13 (8/61)</td>
</tr>
<tr>
<td>1–3</td>
<td>13 (6/64)</td>
<td>0 (0/7)</td>
</tr>
<tr>
<td>4</td>
<td>88 (14/16)</td>
<td>95 (18/19)</td>
</tr>
<tr>
<td>Total</td>
<td>16 (32/206)</td>
<td>30 (26/87)</td>
</tr>
</tbody>
</table>

NOTE. Data are percentage of sustained response (SR) cases (no. SR cases/total cases). aa Residues are indicated by standard single-letter code. ISDR, IFN sensitivity-determining region.

* Between H and C. 
* Between H and C (P = .005), R and C (P = .008), H and N (P = .010), and R and N (P = .021).

* Between H and R (P = .008), H and C (P < .001), H and N, (P = .048), and R and C (P = .017).
TR and NR was similar regardless of the number of mutations (figure 2), which indicates that the ISDR is associated mainly with viral eradication resulting in SR, but not with the transient viral suppression seen in TR.

Viral kinetic studies suggest that there are 2 phases of HCV elimination during IFN treatment: a rapid decline in viral concentration in serum, reflecting blockade of virus production or release, followed by a slower second phase reflecting the rate of death of the infected cells [40]. The first phase is universally observed among HCV-infected patients and causes the transient viral decrease during treatment regardless of the final outcome, whereas the second phase of infected cell death is associated with final eradication of HCV (i.e., SRs). Therefore, HCV with wild- or intermediate-type ISDR might be resistant to the second-phase effect of IFN (i.e., killing of HCV-infected cells, resulting in TR or NR). The NS5A protein inhibits induction of apoptosis by PKR or tumor necrosis factor-α [32, 41]. For complete HCV eradication, in addition to suppression of viral replication, elimination of infected hepatocytes through IFN-induced apoptosis might be mandatory, and possibly this is blocked by competent NS5A with wild- or intermediate-type ISDR. The relationship between ISDR sequences and HCV kinetics under IFN should be studied.

In Japan, most investigators have reported data similar to ours concerning the relationship between ISDR mutations and IFN efficacy. However, in Western countries, there have been conflicting data. Results in our present study definitively confirmed the association between ISDR mutations and IFN effects in Japanese patients. Differences in findings between Japanese and European HCV-1b may be explained by racial or virologic differences and other factors.

First, in Western countries, the mutant type is rare (0%-17%) [7–11], so statistically relevant analysis to evaluate the effect of ISDR mutations is problematic. Second, even patients with mutant-type ISDR may not respond to treatment, because lower doses of IFN are generally used in Western countries than in Japan. One recent study revealed an association between the number of ISDR mutations and IFN efficacy in combination therapy (IFN and ribavirin) [15], which suggests that the association between ISDR and IFN efficacy might indeed become clearer under more intense antiviral treatment. Third, some investigators have categorized SR and TR together as responders [9, 42–44]. However, as shown in this study, the same range of mutations is found in TR and NR, so that analysis that combines SR and TR groups would be inappropriate and perhaps would fail to find any association between ISDR mutations and response to IFN. Fourth, some studies analyzed genotypes 1a and 1b together [10, 44], compared with the reference sequence of HCV-1b. However, in our recent work, we observed a similar association between ISDR and IFN efficacy in genotype 2a but not in genotype 2b [45]. Similarly, Sarrazin et al. [33] reported a correlation between IFN efficacy and the PKR-binding domain but not ISDR in genotype 1a; yet, in our present study, only the ISDR showed correlation in genotype 1b. Thus, the influence of ISDR mutation on IFN efficacy is not uniform among the different genotypes. Moreover, even in genotype 1b, there are 3 groups (non-Japanese, Japanese, and worldwide strains). In Japanese strain HCV-1b, there is a close correlation between the mutation number of ISDR and IFN effect that is not observed in worldwide strain HCV-1b [46]. Of interest, preliminary findings fit these data in another group [47]. Therefore, geographic diversities of HCV genotypes might cause the different ISDR roles seen in IFN therapy in Japan and Western countries.

The present investigation documents different mutational patterns of the ISDR in a large number of infected patients and demonstrates their diverse influences on the efficacy of IFN treatment. These data suggest that the presence of aa residues in the ISDR is critical for NS5A function. Such results will help to elucidate the relationship between the structure of the NS5A protein and its function and provide a means of predicting clinical efficacy of IFN in individual patients.

References


