Sequences in the NS5A protein of hepatitis C virus and the serum alanine aminotransferase response to interferon therapy in Japanese patients

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Abstract

***Background and aims*—Chronic hepatitis C is a slowly progressive disease and eventually causes hepatocellular carcinoma in many patients. Although interferon (IFN) therapy has been used for viral eradication, its success rate is only about 30%. In patients in whom it has failed (non-responders), there are several patterns of serum alanine aminotransferase (ALT) values, and detection of serum HCV-RNA during and after IFN therapy and improved long term prognosis were reported in patients whose serum ALT values were normalised by IFN therapy even if HCV viraemia persisted. The present study sought to clarify the virological characteristics contributing to these differences.**

***Methods*—Complete or partial length dominant sequences of hepatitis C virus genotype 1b (HCV-1b) were determined by direct sequencing. Firstly, the complete sequences of HCV-1b genomes were determined in six non-responders; three showed normalisation of serum ALT values during IFN-*α* therapy and the other three did not. Subsequently, the amino acid residues that were different in the two groups were further analysed retrospectively in another 82 patients.**

***Results*—Comparison of the sequences suggested an association between amino acids 2154–2172 of HCV-1b and serum ALT normalisation. A retrospective analysis of 82 patients revealed that the number of amino acid substitutions in this region was the only statistically significant variable associated with ALT normalisation (odds ratio 31.0; 95% confidence interval 5.0–286) in multivariate analyses.**

***Conclusions*—A HCV genomic region that correlates with the ALT response to IFN therapy appears to be present in virologically IFN ineffective patients.**

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Keywords: hepatitis C virus; alanine aminotransferase; biochemical responder; transient responder, NS5A protein

Hepatitis C virus (HCV) is a single stranded RNA virus consisting of approximately 9500 nucleotides.[1–3] Persistent HCV infection causes chronic hepatitis C, which may develop into liver cirrhosis and hepatocellular carcinoma (HCC) over 20–30 years in some patients.[4] To date, interferon (IFN) alone or combined with ribavirin is the only curative therapy for chronic hepatitis C.[5] However, its efficacy is as low as 30% in patients with HCV genotype 1b infection, the predominant genotype in Japan, even when a sufficient amount of IFN is administrated.[6] HCV viraemia is not cleared in more than half of HCV-1b infected patients, and they continue to be at risk for disease progression. As a result, clinical and virological analyses targeted at these patients is important.

A complete responder (CR) to IFN therapy is defined as a patient negative for serum HCV-RNA, as assayed by reverse transcription-polymerase chain reaction at six months after the final IFN administration.[5] A patient who does not meet this criterion is defined as a non-responder (NR). However, NRs can be divided into subgroups according to serum alanine aminotransferase (ALT) values and/or the presence of HCV-RNA. Figure 1 schematically shows the course of ALT values in NRs. In fig 1A, ALT values are persistently abnormal throughout the course of treatment. This group is defined as biochemical non-responders (bNRs) in this study. In fig 1B, while ALT values transiently normalise during IFN therapy (usually within eight weeks), they return to abnormal levels within six months after cessation of IFN administration. This group is defined as biochemical transient responders (bTRs). In fig 1C, ALT values normalise during IFN therapy and are sustained for more than six months after treatment. This group is a special subgroup of bTR and can be included in bTR, but we define this patient group as biochemical responders (BRs). These different courses between bTR and BR appear to have clinical correlations. Kasahara *et al* reported that patients with the clinical course shown in fig 1B or 1C have a lower risk of developing HCC[7] compared with those showing the course demonstrated in fig 1A. Nishiguchi *et al* also reported that reduced ALT values as a result of IFN therapy led to a decreased incidence of HCC.[8] It has also been reported that the incidence of HCC declines in

Abbreviations used in this paper: IFN, interferon; ALT, alanine aminotransferase; HCV, hepatitis C virus; HCV-1b, HCV genotype 1b; HCC, hepatocellular carcinoma; CR, complete responder; NR, non-responder; BR; biochemical responder; TR, transient responder; ISDR, interferon sensitivity determining region; NCR, non-coding region; PCR, polymerase chain reaction; ARE, ALT response related element; HCV-2a, HCV genotype 2a; HCV-2b, HCV genotype 2b; CTL, cytotoxic T lymphocytes.

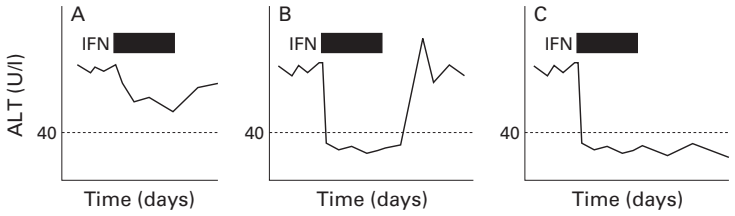


Figure 1 Schematic diagram of the typical clinical courses of serum alanine aminotransferase (ALT) values during and after interferon (IFN) therapy. (A) Patient without ALT normalisation (biochemical non-responder (bNR)); (B) patient with ALT normalisation and subsequent flare up (biochemical transient responder (bTR)); and (C) patient with ALT normalisation and sustained normal ALT values after IFN therapy (biochemical responder (BR)).

Table 1 Clinical backgrounds of the initial six patients

	Normalised ALT group			*Abnormal ALT group*		
	Patient 1	*Patient 2*	*Patient 3*	*Patient 4*	*Patient 5*	*Patient 6*
Age (y)	51	42	61	63	53	58
Sex (M/F)	F	M	M	F	M	M
Pretreatment serum ALT (IU/l)	98	93	129	56	92	106
ALT at final IFN session (IU/l)	10	10	24	47	109	49
Platelet count (×104/mm^3)	13.8	17.4	20.9	19.2	10.5	15.5
HCV-RNA (Meq/ml)*	11	<0.5	11	7.6	15	10
Histological findings†						
Activity	2	1	2	2	2	3
Fibrosis	3	1	2	3	3	3

*HCV-RNA levels were determined by a branched chain DNA assay.
†Histological findings were classified according to the criteria described by Enomoto and colleagues.[12]
ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon.

BR patients.[6] This reduction in HCC incidence implies a better long term prognosis in these patients.

We recently reported that patients who showed transient serum HCV-RNA disappearance during and shortly after (less than six months) IFN therapy (we define such patients as virological transient responders (vTRs)) showed a lower incidence of HCC compared with patients who did not experience HCV-RNA disappearance during and after IFN therapy (virological non-responders (vNRs))(N Izumi *et al*, unpublished observation). As a possible explanation for these observations, Poynard *et al* showed that IFN therapy significantly reduced the grade of inflammatory activity and stage of fibrosis,[9] resulting in a lowering of the risk of developing HCC. In this study, we attempted to elucidate the viral determinant(s) associated with bTRs because it may be related to the preferable prognosis of IFN therapy.

In our previous studies we have shown that IFN resistant patients can be predicted by the substitution number in the IFN sensitivity determining region (ISDR)[10 11] but virological factors concerning differences in the pattern of ALT fluctuation in NRs have not been determined. Thus we analysed the complete genomes of HCV to elucidate any virological factors that may be associated with different patterns of ALT fluctuation.

Patients and methods

PATIENTS

We studied 88 patients with chronic HCV-1b infection. All were positive for serum anti-HCV antibodies (third generation assay) and serum HCV-RNA of genotype-1b.[12 13] Serum HCV-RNA levels were determined by a branched chain DNA assay[14] (Quantiplex HCV-RNA, Chiron, Emeryville, California, USA). The limit of detection of the assay was 0.5 million genome equivalents/ml. Patients were negative for serum HBs antigen, anti-HBc antibodies, and antinuclear antibodies, and had no other causes of hepatitis, including excessive alcohol intake or hepatotoxic drugs. Liver biopsies were performed in all patients, and the presence of chronic active hepatitis was confirmed histologically. Written informed consent was obtained from each patient for liver biopsy, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Institutional approval was obtained.

Initially, full length sequences of HCV were determined in six patients. All six received IFN therapy but were found to be NRs. Each serum sample was obtained just before the start of IFN therapy. Among them, three showed persistently elevated serum ALT values during and after IFN therapy (similar to the clinical course shown in fig 1A) (elevated ALT group), and in the other three, serum ALT values normalised within eight weeks after the start of IFN therapy, similar to the clinical course shown in fig 1B (normalised ALT group). The clinical backgrounds of the patients are shown in table 1. The six full length sequences were aligned, and amino acid (for the coding region) or nucleotide (for the non-coding region (NCR)s) usage was compared between the two groups. Only residues in which amino acid (or nucleotide) usages were completely different were regarded as being possibly associated with the divergent clinical courses.

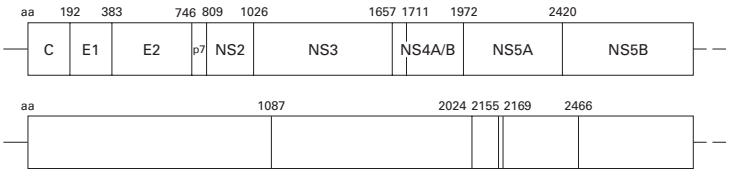
Subsequently, the sequences of selected residues were determined in another 28 patients, and only regions in which amino acid usage was significantly different between the elevated and normalised ALT groups were further analysed. Finally, the relationship between the amino acid sequence and clinical course was retrospectively analysed in 82 patients.

RNA EXTRACTION

Experimental methods were conducted as described previously.[15 16] Serum RNA was extracted by the acid-guanidium-phenol-chloroform method. Briefly, 150 µl of serum were mixed with 700 µl of ISOGEN (Wako Pure Chemical Industries, Osaka, Japan), and the aqueous phase was extracted once with 140 µl of chloroform. RNA was isopropanol precipitated with 20 µg of glycogen (Boehringer Mannheim, Manheim, Germany) as a carrier. The resultant RNA was washed once with ethanol and finally dissolved in 10 µl of water and stored at −70°C until use.

cDNA SYNTHESIS

A 5 µl reaction volume contained 1 µl of the RNA solution, 50 U of reverse transcriptase (M-MLV Reverse Transcriptase; Life Technologies Inc., Gaithersburg, Maryland, USA) and its accompanying buffer, 10 units of RNase



Amino acid (aa)	1087	2024	2155	2169	2466
Normalised ALT group (n = 3)	A	V	P	T	Q
Elevated ALT group (n = 3)	S	I	L	A	L
HCV-J (reference sequence)	S	I	L	A	L

Figure 2 Codons for which the encoded amino acid are different between the two groups. The upper column shows the architecture of the hepatitis C virus (HCV) polyprotein and the lower column shows the distribution of the amino acid residues. Amino acid usages in each group and HCV-J are shown in the lowermost table. Amino acids are described in the single letter code.

inhibitor (Promega Corp., Madison, Wisconsin, USA), and 50 pg of random hexamers (Takara, Kyoto, Japan). The mixture was incubated at 37°C for 40 minutes.

POLYMERASE CHAIN REACTION (PCR)

The full length HCV genome was amplified by nested PCR with 20 partially overlapping primer sets.[10 15] We used a hot start PCR technique with wax (Ampliwax PCR Gem 50; Takara). The lower solution contained 1.5 mM $MgCl_2$, 400 nM of each PCR primer, and 200 μM of each deoxyribonucleoside triphosphate. In the upper solution, 1 μl of the cDNA solution was dissolved in 50 μl of a PCR mixture containing 10 mM tris HCl (pH 8.3), 0.01% gelatin, and 0.5 U of Taq polymerase (AmpliTaq; Promega Corp.).

SEQUENCE DETERMINATION

Each PCR product was purified and excess primers were excluded by a column (Suprec 02; Takara) according to the manufacturer's instructions. Thereafter, both strands of the PCR products were cycle sequenced with the PRISM dye termination kit (Applied Biosystems, Chiba, Japan) according to the manufacturer's instructions. The sequencing primers were an M13 forward primer for the sense strand and an M13 reverse primer for the antisense strand. The products were purified on a column (Quickspin column; Boehringer Mannheim, Indianapolis, Indiana, USA) and sequenced using an automated DNA sequencer (model 373S; Applied Biosystems). The nucleotide and deduced amino acid sequences were compared using the sequence of HCV-J[17] as a reference.

STATISTICAL ANALYSIS

The χ^2 test or Fisher's exact test was used for comparison of amino acid usage, as appropriate (Statview-J v. 4.5; Hulinks Inc., Tokyo, Japan). Statistical analyses of the relationship between clinical parameters and amino acid sequences were performed by logistic regression analyses (univariate or multiple as appropriate) with the JMP program (v. 3.1, SAS Institute, Cary, North Carolina, USA).

Results

COMPARISON OF THE FULL LENGTH HCV SEQUENCES

Codons for which the encoded amino acids were completely different between the two groups are shown in fig 2. Complete amino acid usage differences were observed in only five residues. Serine, isoleucine, leucine, alanine, and leucine were used in amino acids 1087, 2024, 2155, 2169, and 2466, respectively, in the elevated ALT group; these were the same as those in HCV-J. However, alanine, valine, proline, threonine, and glutamine, respectively, were used in the normalised ALT group. No differences in nucleotide usage were observed in NCRs.

Subsequently, amino acid sequences around these five residues were determined in 28 NRs. Among them, 16 belonged to the elevated ALT group (bNR) and 12 to the normalised ALT group (bTR). Amino acid usages were significantly different between the two groups at amino acids 2155 ($p=0.022$ by χ^2 test, proline for bTR and leucine for bNR) and 2169 ($p=0.050$ by Fisher's exact test, threonine for bTR and alanine for bNR). However, no significant difference was seen at amino acids 1087 ($p>0.99$ by Fisher's exact test, alanine for bTR and serine for bNR), 2024 ($p=0.11$ by Fisher's exact test, valine for bTR and isoleucine for bNR), or 2466 ($p=0.13$ by χ^2 test, glutamine for bTR and leucine for bNR). Besides these residues, amino acid usage was also different at amino acid 2171 ($p=0.044$ by Fisher's exact test, non-leucine for bTR and leucine for bNR). Thus the predicted amino acid sequences in the region from amino acids 2154 to 2172 appeared to show a correlation with the course of serum ALT values during IFN therapy. We propose to name this region the ALT response related element (ARE). HCV from the normalised ALT group had more amino acid substitutions in this region compared with HCV-J while HCV from the elevated ALT group had fewer. From these preliminary results, we explored the relationship between the course of ALT values during and after IFN therapy and the sequence of the ARE in larger numbers of patients.

ANALYSIS OF THE RELATIONSHIP BETWEEN THE ALT RESPONSE AND THE ARE

In total, 82 patients were included in the analysis. All patients received IFN therapy and were judged to be NRs. Among the 82 patients, 25 belonged to the elevated ALT group (bNR) and 57 to the normalised ALT group (bTR). In the normalised group, 23 met the criteria for BR; all BR patients showed ALT normalisation during IFN treatment. Clinical backgrounds of the patients in each group are summarised in table 2. Figure 3 demonstrates the relationship between ALT response and the sequence of the ARE. Amino acid substitution seemed to be permitted only at amino acids 2154, 2155, 2169, and 2171. As the number of amino acids that are different from those of HCV-1b (defined as "substitution number") increases, it is more likely that an ALT response is obtained. Figure 4 shows the relationship

Table 2 Clinical backgrounds of the 82 patients

Variable	
Age (y)	47.7 (10.3)
Sex (M/F)	56 / 26
Pretreatment serum ALT (U/l)	100.2 (59.6)
F factor	1.80 (0.74)
Serum HCV-RNA load (Meq/ml)	5.0 [<0.5–40]
Mutation number in ISDR	1.0 [0–6]

Values are number, mean (SD), or median [range].
ALT, alanine aminotransferase; HCV, hepatitis C virus; ISDR, interferon sensitivity determining region.

between the rate of ALT normalisation and substitution number in the ARE. The substitution number ranged from 0 to 4. Clearly, ALT response rates are positively correlated with the substitution number in the ARE ($p=0.004$ on simple χ^2 test).

Additionally, the relationship between substitution number in the ARE and vTR—that is, the disappearance of serum HCV-RNA—was analysed. While bTR frequently coincided with vTR ($p<0.0001$, χ^2 test), dissociation between the two phenomena was also observed (table 3) in some patients. In particular, bTR preceded BR in all 23 BR patients (by definition) while vTR preceded BR in only 17 patients.

Factors possibly associated with ALT normalisation were analysed by univariate analysis with substitution number in the ARE, substitution number in the ISDR, serum HCV-RNA titre, age, F factor (according to international criteria[18]), and pretreatment serum ALT value as independent variables (table 4A). Using this analysis, only the substitution number in the ARE was extracted as a statistically significant factor ($p=0.0003$). Subsequent multiple logistic regression analysis revealed that the substitution number in the ARE was the only independent factor for normalisation of ALT ($p=0.0007$) (table 4B). Of the above mentioned variables, only the substitution number in the ISDR correlated with the substitution number in the ARE ($p=0.003$). The relationship between substitution number in the ARE and BR did not reach statistical significance.

Finally, the substitution numbers in the ARE were determined in 11 patients who received IFN therapy and were CRs. However, no relationship was found between serum ALT values during IFN treatment and substitution number in the ARE. The substitution number in the ARE also ranged from 0 to 4 in this group. The amino acid sequences of the ARE in HCV-2a (corresponding to amino acids 2158 to 2176 of HC-J6; five amino acid substitutions are present compared with HCV-J) and HCV-2b (corresponding to amino acids 2158 to 2176 HC-J8; six amino acid substitutions are present compared with HCV-J) were also determined in six patients with HCV-2a infection and 10 with HCV-2b, all of whom received IFN therapy and were NRs but showed ALT normalisation during IFN therapy and were TRs. However, only one amino acid substitution was observed in HCV-2a (T2176M) in five of six patients, and no amino acid polymorphism was observed in this region of HCV-2b.

Discussion

In this study, the relationship between the pattern of ALT response to IFN therapy and HCV sequences was analysed. We found that amino acid substitutions in NS5A (amino acids 2154–2172, a region we have named the ALT response related element (ARE)) that differ from the prototype HCV-1b sequence, were strongly associated with normalisation of ALT during IFN therapy.

Variations in viral genomic sequence and clinical presentation have been associated with some RNA viruses, such as pestiviridae (including HCV) and flaviviridae. In encephalitogenic flaviviruses, such as yellow fever virus, Japanese encephalitis virus, or tick borne encephalitis virus, attenuation of passaged viruses was consistently associated with amino acid changes in protein E, and a single amino acid change in a critical determinant of protein E can be sufficient for loss of neuroinvasiveness.[19] However, studies addressing this issue in HCV are rare. We have previously shown in the natural course of chronic hepatitis C that time related amino acid substitutions in the NS5A/NS5B protein of HCV are associated with changes in the activity of hepatitis[15]; this is the only report of which we are aware associating HCV genomic sequences with activity of hepatitis. Similarly, the present study shows a relationship between HCV sequences and the course of serum ALT levels, which reflects hepatitis activity during IFN treatment. To date, differences in hepatitis activity during the natural course of chronic hepatitis C in different patients was analysed mainly from the point of view of the genetic background of the host. For example, HLA-DR5 was reported to be associated with anti-HCV positive symptom free individuals,[20] and TAP2*0103 (transporter associated with antigen processing 2) has been associated with patients with persistently normal ALT values.[21] However, our results indicate that viral sequences may also be important.

We have previously characterised the ISDR sequence at positions 2209–2248 of the NS5A protein of HCV as a virological determinant predicting the efficacy of IFN therapy.[10 11] Although the genomic region of the ARE is different from that of the ISDR, the ISDR sequence in HCV is also associated with a clinical presentation of chronic hepatitis C. In Japanese patients, the ISDR sequence is useful for predicting whether a complete response will be achieved[11] although there appears to be some controversy. Similarly, the ARE sequence may be useful for predicting whether ALT normalisation will occur, although a prospective study is necessary to establish this.

The ISDR sequence is located about 60 amino acids downstream of the ARE, and the number of amino acid substitutions in these two regions was correlated. Although it cannot be concluded from this study that there are any functional relationships between the ARE and the ISDR, it is possible and they may operate together. While Duverlie *et al* recently suggested that substitution at amino acid 2169 is related to sensitivity to IFN therapy,[22] we could

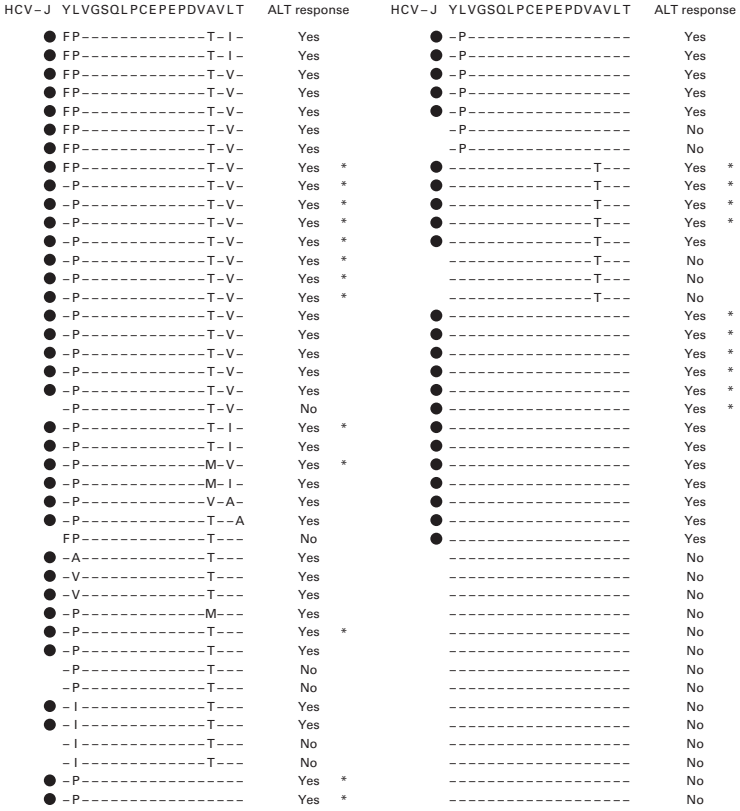


*Figure 3 Relationship between amino acid sequences in the alanine aminotransferase (ALT) response related element (ARE) in 82 patients. Amino acid residues from 2154 to 2172 are shown. Amino acid residues identical to those of HCV-J are shown as "–". Filled circles (●) indicates that serum ALT values normalised during interferon therapy. *Biochemical responders.*

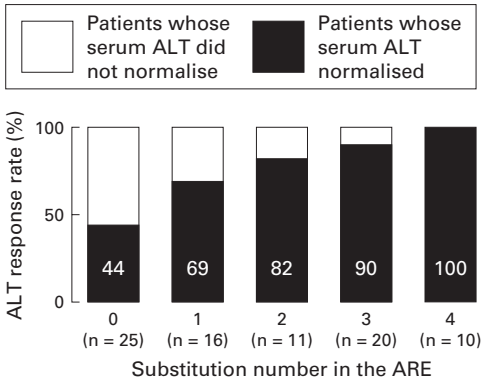


Figure 4 Relationship between substitution number in the alanine aminotransferase (ALT) response related element (ARE) and the proportion of patients with ALT normalisation. The number of patients in each group is shown in parentheses.

not confirm this finding in the present study because no relationship between the substitution number in the ARE and ALT response in CRs was found. There may be some differences in HCV between Japanese and European strains,[23] and further studies will be needed to determine any relationship between the ARE and the ISDR.

To date, the function of the peptide encoded by the ARE and adjacent regions has not been fully elucidated. The ARE sequence is located in the acidic domain 1, which is important for the transactivator activity of NS5A.[24–26] Although the functional significance of amino acid substitutions in the ARE sequence awaits further in vitro study, the L2155P substitution may have some impact on transactivator activity because it alters the polarity of the amino acid. Indeed, we have shown that amino acid mutations in the acidic domains attenuate the transactivator activity of the NS5A.[24] Another study suggested that there is a possible alternative processing site of the NS5A protein at amino acid 2172[27] by NS3 protease. Processing at this site produces a truncated NS5A protein that can translocate into the nucleus and exhibit transactivator activity. Substitutions at amino acids 2169, 2171, and 2172 may alter the processing efficiency and influence this putative NS5A function.

Table 3 Relationships between alanine aminotransferase (ALT) response and virological transient responder (vTR) among the 82 patients who received interferon therapy (number of patients)

	vTR (n=50)	*vNR (n=32)*
ALT normalisation (n)		
bTR (59)	44	15
BR (23)	17	6
bNR (23)	6	17

$p<0.0001$, χ^2 test (total bTR *v* bNR).
vNR, virological non-responders; bTR, biochemical transient responder; BR, biochemical responder; bNR, biochemical non-responder.

There was a strong correlation between bTR during IFN therapy and vTR, although inconsistent cases were also observed. While the relationship between substitutions in the ARE and bTR seemed to be more direct than vTR, the collinearity of bTR and vTR made a multivariate analysis difficult for discrimination; a larger number of samples are needed to address this point.

Alternatively, profound inhibition of HCV-RNA replication in hepatic tissue during IFN administration may cause suppression of hepatocellular injury resulting in decreased ALT levels. If so, the ARE may determine the efficacy of HCV replication during IFN treatment. Hepatocyte injury in chronic hepatitis C is thought to be caused by cytotoxic T lymphocytes (CTL) against virus derived peptide presented on the hepatocyte surface in HLA molecules. Therefore, profound suppression of HCV replication by IFN may cause the disappearance of such viral epitopes resulting in a decrease in hepatocyte injury. Also, the ARE may directly determine the activity of hepatitis during IFN therapy. For example, a specific ARE sequence could modulate HLA expression or peptide presentation, which is usually enhanced by IFN, resulting in decreased hepatocellular damage by CTL. In fact, a recent report suggested that polymorphism in a peptide transporter involved in antigen presentation is associated with hepatitis activity.[22]

It has been suggested that the long term prognosis may improve, even in patients who did not become CRs with IFN therapy. Kasahara *et al* showed in a cohort study of 1022 patients that the cumulative incidence of HCC was significantly higher in those patients who showed persistently abnormal ALT values.[7] A

Table 4 Factors contributing to the alanine aminotransferase (ALT) response to interferon therapy

Variable	*p Value*	*Odds ratio*	*95% CI*
(A) Univariate analysis			
Mutation numbers in the ARE	0.0003	37.4	6.3–333
Mutation numbers in the ISDR	0.078	59.7	1.02–9300
Pretreatment serum HCV-RNA (Meq/ml)	0.128	0.22	0.04–1.53
Age	0.177	0.23	0.02–1.86
F factor	0.250	0.47	0.12–1.73
Pretreatment serum ALT value(IU/l)	0.492	0.48	0.06–4.23
(B) Multivariate analysis			
Mutation numbers in the ARE	0.0007	31.0	5.03–286
Mutation numbers in the ISDR	0.378	5.30	0.23–471
Pretreatment serum HCV-RNA (Meq/ml)	0.191	0.17	0.01–2.26
Age	0.195	0.25	0.03–2.00

ISDR, interferon sensitivity determining region; ARE, ALT response related element; HCV, hepatitis C virus; CI, confidence inerval.

follow up study of a large number of patients[6] demonstrated a reduced incidence rate of HCC in BRs. More recently, we showed that patients who were vTRs also had a lower probability of developing HCC in a prospective analysis. These findings suggest that the benefit of IFN therapy may not be observed only in BRs (those whose ALT course resembles fig 1C), but also in bTRs who do not fulfill the criteria for BR (those whose ALT course resembles fig 1B). This long term effect of IFN therapy may be secondary to a decrease in the progression of liver disease by reducing the grade of inflammatory activity and stage of fibrosis[8 28] or direct ablation of precancerous cells by an anti-oncogenic effect of interferon regulatory factor 1 induced by IFN.[28]

Although re-biopsy after IFN therapy was not performed in this study, it is reasonable to postulate that the reduction in inflammatory activity also occurred in our subjects showing normalised serum ALT values following IFN treatment. As a consequence, a virological marker to identify a group of patients who have a better long term prognosis after IFN therapy was suggested in this study, which is effective even in those whose serum HCV could not be eradicated by IFN.

In conclusion, a sequence element (the ARE) in the NS5A protein of HCV-1b was identified that was significantly associated with ALT normalisation or transient serum HCV-RNA disappearance during IFN therapy in those who did not become CRs. The possibility that patients with substitutions in the ARE may have a better long term prognosis was suggested, and indicates that the ARE sequence could be a useful marker for selection of candidates for IFN therapy. Although the biological significance of the mutations in the ARE is currently unknown, an explanation may contribute to our understanding of the underlying mechanism causing hepatocellular injury in chronic hepatitis C.

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