TABLE II. Sequence of Primers

Code	Primer sequence	Position (HBV)	Note
MM6	5′-AGGTATGTTGCCCGTTTGTCCT-3′	459–480	HBV S
MM7	5′-CAGCAAAGCCCAAAAGACCCAC-3′	1001–980	HBV S
MM37	5′-TGCCAAGTGTTTGCTGACGC-3′	1174–1193	HBV X
MM26	5′-GTTCACGGTGGTCTCCATG-3′	1627–1609	HBV X
HW1	5′-CCGGAAAGCTT GAGCTCTTCTTTTTCACCTCTGCCTAAT-3′	1818–1839	HBV core
MM31	5′-AGTGCGAATCCACACTC-3′	2288–2269	HBV core
MM24	5′-TGCCAACTGGATCCTTCGCGGGACGTCCTT-3′	1392–1423	ccc Ditection (1st)
MM25	5′ GGAAGGAAAGAAGTCAGAAGG-3′	1978–1960	ccc Ditection (2nd)
HW2	5′-CCGGAAAGCTT GAGCTCTTCAAAAAGTTGCATGGTGCTG-3′	1826–1807	ccc Ditection
UP5	5′-CAGUGCCAAGUGUUUGCUGACGCCAAAGUGCUGGGAUUA-3′		Alu-sense
T3-515	5′-AUUAACCCUCACUAAAGCCUCGAUAGAUYRYRCCAYUGCAC-3′		Alu-antisense
UP6	5′-CAAGTGTTTGCTGACGCCAAAG-3′		Alu-sense (tag)
midT3	5′-ATTAACCCTCACTAAAGCCTCG-3′		Alu-antisense (tag)
pUTP	5′-ACAUGAACCUUUACCCCGUUGC-3′	1131–1152	HB1 (HBV X)
MM37			HB2 (HBV X)
MM60	5′-CTGCCGATCCATACTGCGGAAC-3′	1258–1279	HB3 (HBV X)
uPre 31	5′-GAGUUCUUCUUCUAGGGGACCUG-3′	2350–2328	HB1 (HBV core)
MM31			HB2 (HBV core)
MM25			HB3 (HBV core)

Numbering of nucleotides is according to Ono et al. [1983].
Underlined bases represent added restriction enzyme sites. U = dUTP; Y = (C,T); R = (A,G).

to hemi-nested PCR with the initial primers to obtain discrete bands.

Amplified PCR products were analysed by electrophoresis on 1.0% agarose gel and transferred to a Hybond-N$^+$ nylon membrane (Amersham-Pharmacia, Buckinghamshire, UK). To prepare a total HBV probe, the total HBV genome was amplified according to the method of Günther et al. [1995], and the HBV-specific-bands were detected by hybridisation with a DIG-labelled probe (Roche).

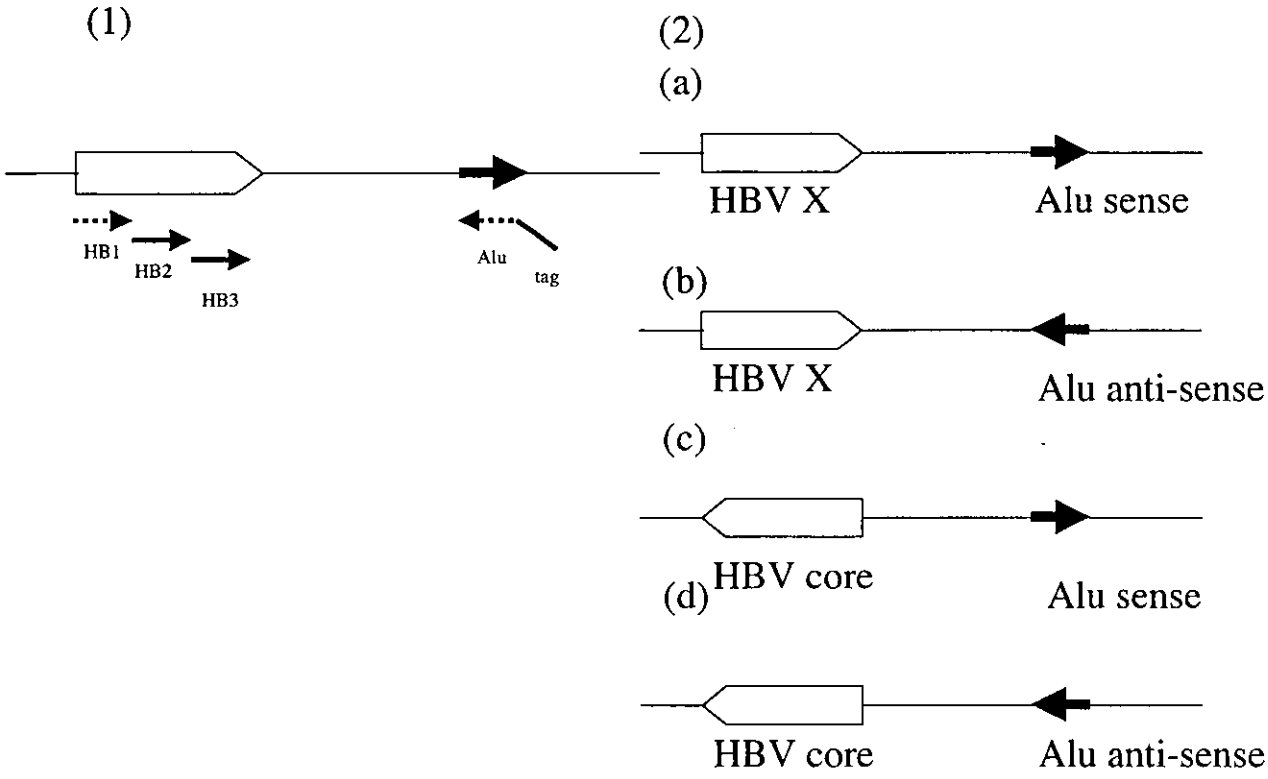

Fig. 1. 1: Technical protocol for the detection of the viral–host junction. The small broken arrows and small intact arrows represent the primer that synthesizes dUTP instead of dTTP and the usual primer, respectively. 2: Four Alu–PCR primer pairs. The locations of the primers are as follows: HBV X region and Alu-sense, HBV X region and Alu-antisense, HBV core region and Alu-sense, and HBV core region and Alu-antisense. The open boxes, lines, and large arrows represent the integrated HBV sequence, genomic sequence, and Alu sequence, respectively.

Direct sequencing. The sequences of the viral host junction were determined from the PCR products using the dideoxy chain termination method. After removing excess primer and deoxyribonucleotides using an Easy trap kit (Takara, Otsu, Japan), the DNA was subjected to a sequencing reaction using the Prism Taq DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems, Inc., Foster City, CA), according to the manufacturer's instructions. The sequencing products were precipitated with ethanol and then subjected to electrophoretic separation and analysis with a 377 Prism DNA sequencer (Applied Biosystems, Inc.).

Detection of Covalently Closed Circular (ccc) HBV DNA

The amplification method used in this study was similar to that employed to identify ccc duck hepatitis B virus (DHBV) DNA [Mason et al., 1998]. A 100 ng sample of genomic DNA was amplified in a 50 μl reaction mixture containing 10 pM of each primer (MM24 and MM25) and 2.5 U of rTaq DNA polymerase (Toyobo). Thirty-five cycles of amplification were performed (94°C for 30 sec, 53°C for 30 sec, and 72°C for 2 min), with an initial denaturation at 94°C for 1 min and a final extension at 72°C for 10 min in a thermal cycler. One microliter of the first PCR product was amplified with 10 pM of hemi-nested primer (MM24 and HW2).

RESULTS

Sensitivity of PCR and Alu–PCR

To assess the sensitivity of the PCR technique, cloned HBV DNA was diluted serially with double distilled water. A mixture of HBV DNA diluted serially and 100 ng of human genomic DNA from the peripheral blood mononuclear cells (PBMCs) of control subjects without any history of liver disease was used as the template. The fifth dilution (10^{-2} pg/100 ng of genomic DNA) was detected using each primer pair (HBV-core, S, and X regions) (data not shown). Alu–PCR sensitivity was determined using a mixture of the DNA from the hepatoma cell line Huh2, containing one copy of integrated HBV DNA per cell [Koike et al., 1983] and human genomic DNA. A 10-fold serial dilution of Huh2 DNA was also prepared; the third dilution (10^2 cells) was detected. Thus, the method can be used to detect 100 copies of integrated HBV DNA sequence in a reaction mixture (data not shown).

Detection and Sequence Analysis of Integrated HBV DNA in the Liver Tissues During Acute Infection

HBV DNA integration was detected by Alu–PCR in the liver cells from two of the eight patients with acute hepatitis (patients 4, 8; Fig. 2). The structures of the viral–host junctions and the cellular flanking sequence were examined using the BLAST search system. In case 8, HBV DNA was integrated into the intronic sequence of the tumour necrosis factor (TNF)-induced protein gene (between the second and third exon) (Accession no.: XM090935.1), while in case 4, the HBV DNA was integrated into a repetitive sequence. HBV DNA integration was detected in one of the three patients with subacute fulminant hepatitis (case 1); this patient had two viral–host junctions. In one, HBV DNA was integrated into the intronic sequence of a hypothetical protein gene (LOC169443) (between fifth and sixth exon); in the other, it was integrated into a repetitive sequence (Fig. 3a). HBV DNA integration was not detected in any of the other 8 patients with fulminant hepatitis (Table III).

Detection of HBV DNA Integration in the Liver Tissue of Patients With HBV-Related Chronic Active Hepatitis and HCV-Related Chronic Active Hepatitis

HBV DNA integration was demonstrated in the liver of all 12 patients with HBV-related chronic active hepatitis using Alu–PCR (Table III). Several hybridised bands of polyclonal integration were observed as a smear on autoradiography (Fig. 2). Two of the 10 patients with HCV-related chronic active hepatitis (cases 3 and 6) also exhibited HBV DNA integration (Table III).

Detection of HBV DNA in PBMCs

HBV DNA integration in PBMCs was investigated in 17 patients (7 with HBV-related chronic active hepatitis and 10 with HCV-related chronic active hepatitis). HBV DNA integration was detected in the PBMCs from four of the HBV-related chronic active hepatitis patients (cases 1, 3, 6, and 7) (Table IV and Fig. 3) and from two of the HCV-related chronic active hepatitis patients (cases 4 and 6). The structure of the viral–host junctions was examined using the BLAST search system. In HBV-related chronic active hepatitis (case 1), the HBV DNA was integrated 73 kb downstream of the inositol 1,4,5-triphosphate 3-kinase B (ITPKB) gene (Accession no.: NM002221.1). In HBV-related chronic active hepatitis (case 6a), the viral genome was integrated into the intronic sequence of the gene for an unnamed protein (LOC144362) between eighth and ninth exon (Accession no.: XM038659.3). In HBV-related chronic active hepatitis (case 7), the HBV DNA was integrated 4 kb down stream of a member of the RAS oncogene family (RAB30) (Accession no.: XM006025.4). In HCV-related chronic active hepatitis (case 6), the HBV DNA was integrated into the intronic sequence of the gene for a hypothetical protein (FLJ 12303) between the second and third exon (Accession no.: XM052594.1) (Table III). In the remaining cases, the viral DNA was integrated repetitive sequences. One HCV-related chronic active hepatitis patient (case 4) showed a rearranged viral sequence, and the host junction DNA sequence showed no homology to the EMBL/Genebank sequences (Fig. 3b).

Forms of HBV DNA in Serum, Liver, and PBMCs

Three primer pairs (for the core, S, and X regions) were used to detect HBV DNA. In all seven HBV-related

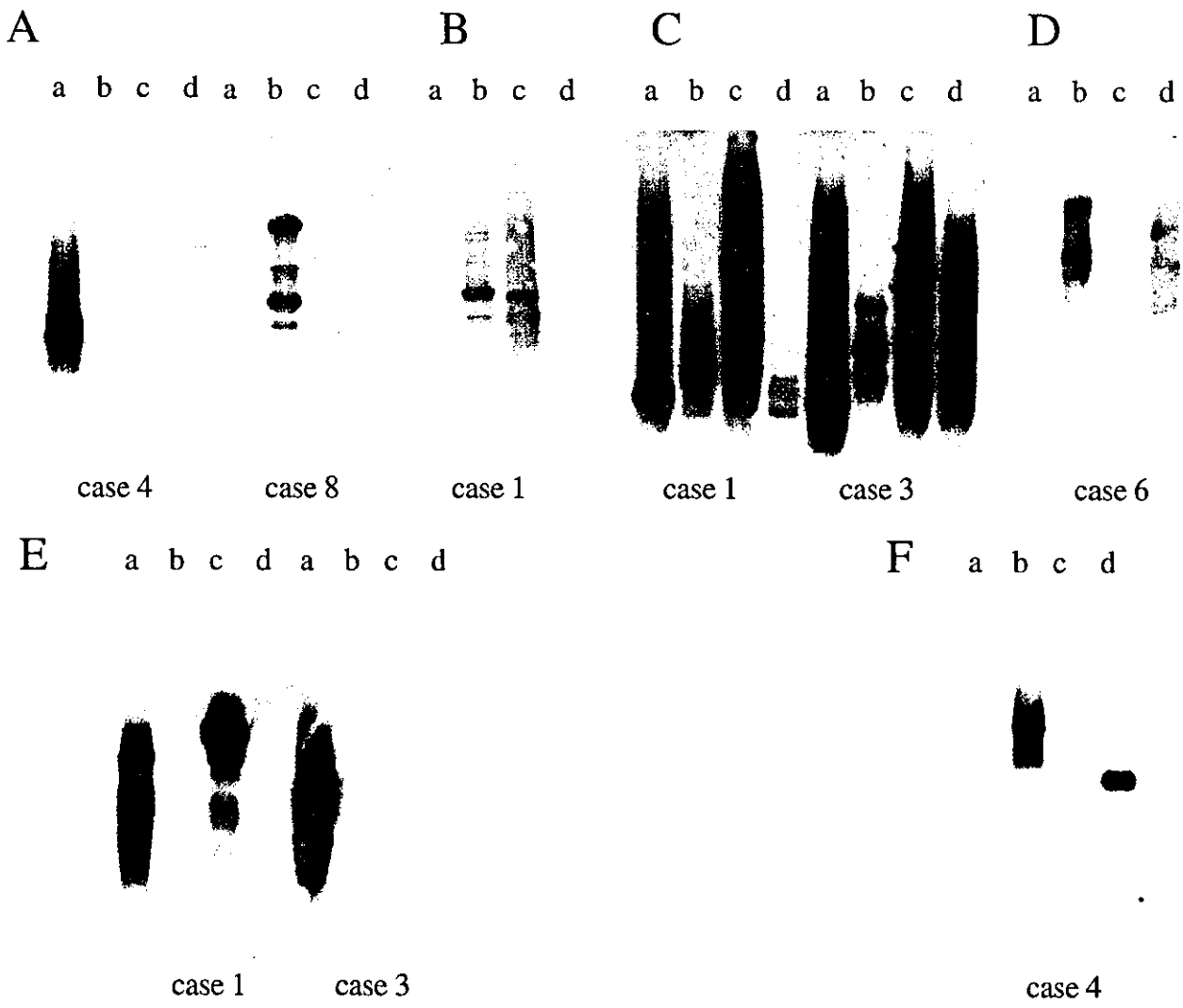

Fig. 2. Detection of viral–host junctions by Alu–PCR. **A–D**: The HBV hybridised bands in liver specimens from acute hepatitis, subacute fulminant hepatitis, HBV-related chronic active hepatitis, and HCV-related chronic active hepatitis patients, respectively. **E** and **F**: The HBV hybridised bands in PBMCs from HBV-related chronic active hepatitis, and HCV-related chronic active hepatitis patients, respectively. **Lanes a, b, c,** and **d** represent the primer pairs corresponding to Figure 1.

chronic active hepatitis patients, HBV DNA was detected in the serum using all three-primer sets. HBV DNA was also detected in the PBMCs of all of these patients. However, HBV DNA was only detected using one or two primer sets in four cases of PBMCs (case 2, 3, 5, and 6); the other three cases had detectable levels of HBV DNA using all primer sets. Among the HCV-related chronic active hepatitis patients, HBV DNA was detected using all three primers sets in sera from cases 2 and 7, in liver tissue from cases 1, 2, 5, 7, and 9, and in PBMCs from cases 2 and 9. HBV DNA was only identified using one or two primer sets in the other cases (Table IV).

Finally, the presence of covalently closed circular (ccc) HBV DNA was examined in the serum, liver, and PBMC samples. ccc HBV DNA was detected in the liver tissue of all seven patients with HBV-related chronic active hepatitis and in one patient (case 1) with HCV-related chronic active hepatitis, but ccc HBV DNA was not detected in the PBMCs from four of the seven patients with HBV-related chronic active hepatitis (cases 2, 3, 4, and 6) or any of the 10 patients with HCV-related chronic active hepatitis, nor was it detected in any of the sera samples (Table IV).

DISCUSSION

Alu–PCR is more sensitive for detecting viral–host junctions than Southern hybridisation and has several other advantages as well. This PCR-based method can effectively amplify the viral–host junction in small samples, such as liver tissue specimens obtained by needle biopsy. This PCR reaction can also be carried out in a single tube and without ligation; as a result, there is less chance of cross-contamination. The Alu-specific and HBV-specific primers provide a fragment containing a hybrid of the viral and cellular sequence, and nonspecific amplification of viral or host sequences can be avoided.

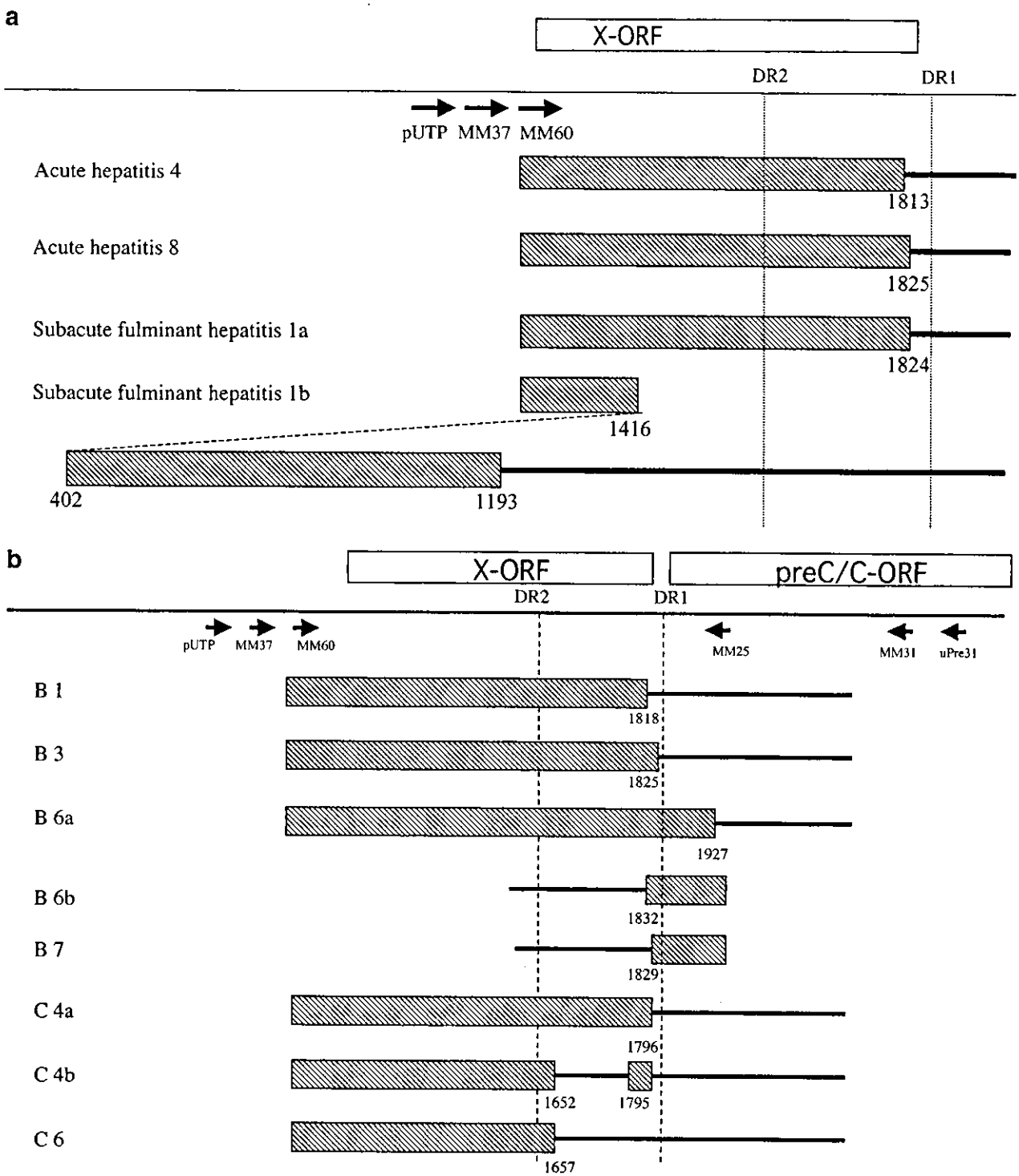

Fig. 3. Structure analysis of the viral–host junctions in liver (a) and PBMCs (b). B and C: HBV-related chronic active hepatitis and HCV-related chronic active hepatitis, respectively. The open boxes represent the HBV open reading frame. X and preC/C correspond to HBV X and precore/core region, respectively. The hatched box, thick line, and arrow represent the HBV sequence, cellular flanking sequence, and primer, respectively. The dotted lines represent rearrangements of the HBV sequence. The nucleotide numbers of the virus pointing at the junctions are presented under the sequence, numbering from the hypothetical EcoRI site of subtype adr [Ono et al., 1983].

The detection limit of this method is about 100 copies of the same viral–host junction per reaction. Positive results for integrated HBV DNA with this method indicate the presence of more than 100 clonally expanded cells with HBV DNA integration.

The present study provides the first definite demonstration of viral DNA integration during the acute stage of HBV infection, both in liver tissues and peripheral blood mononuclear cells (PBMCs). The rates of viral integration detection in acute hepatitis, HBV-related

TABLE III. Summary of HBV DNA Integration

Tissue	Clinical diagnosis	Case no.	HBV integration	Gene/type of sequence	Accession no.	Supercontig (position)	Orientation	Chromosomal localisation
Liver	Fulminant hepatitis	1	−					
		2	−					
		3	−					
		4	−					
		5	−					
		6	−					
		7	−					
		8	−					
	Subacute fulminant hepatitis	1a	+	Intronic sequence of hypothetical protein (LOC 169443)	XM_095704.1	NT_017568.8 (642470)	Same	9q33
		1b	+	Repetitive sequence				
		2	−					
		3	−					
			−					
	Acute hepatitis	1	−					
		2	−					
		3	+	Repetitive sequence				
		4	−					
		5	−					
		6	−					
		7	−					
		8	+	Intronic sequence of TNF-induced protein	XM_090935.1	NT_010194.8 (1832076)	Opposite	15q15.3
	HBV-related chronic active hepatitis	1	+	N.I.				
		2	+	N.I.				
		3	+	N.I.				
		4	+	N.I.				
		5	+	N.I.				
		6	+	N.I.				
		7	+	N.I.				
		8	+	N.I.				
		9	+	N.I.				
		10	+	N.I.				
		11	+	N.I.				
		12	+	N.I.				

	HCV-related chronic active hepatitis	1	−					
		2	−					
		3	+	N.I.				
		4	−					
		5	−					
		6	+	N.I.				
		7	−					
		8	−					
		9	−					
		10	−					
PBMC	HBV-related chronic active hepatitis	1	+	Repetitive sequence				
		2	−					
		3	+	73 kb Downstream of the ITPKB gene	NM_002221.1	NT_004525.8 (736776)	Opposite	1q32
		4	−					
		5	−					
		6a	+	Unnamed protein	XM_038659.3	NT_009471.8 (875218)	Opposite	12p11.1
		6b	+	Repetitive sequence				
		7	+	Intronic sequence of RAB30 gene	XM_006025.4	NT_030106.3 (4012261)	Opposite	11q12
	HCV-related chronic active hepatitis	1	−					
		2	−					
		3	−					
		4a	+	Repetitive sequence				
		4b	+	Repetitive sequence				
		5	−					
		6	+	Intronic sequnece of hypothetical protein (FLJ12303)	XM_052594.1	NT_009538.8 (117792)	Opposite	12q12
		7	−					
		8	−					
		9	−					
		10	−					

Supercontig, homology sequence to human genome; position, HBV DNA integration site in the supercontig sequence; orientation, gene orientation as compared to the direction of the HBV sequence. ITPKB, inositol 1,4,5-triphosphate 3-kinase B; RAB30, member of RAS oncogene family; N.I, no information; SFH-1 (liver), CAHB-6 (PBMC), and CAHC-4 (PBMC) have two integrants.

TABLE IV. Form of the HBV DNA in Serum, Liver Tissue, and PBMCs

		Serum			Liver					PBMCs				
Clinical diagnosis	Case no.	C	X	S	Integration	C	X	X	ccc	Integration	C	X	X	ccc
HBV-related chronic active hepatitis	1	+	+	+	+	NT	NT	NT	+	+	+	+	+	+
	2	+	+	+	+	NT	NT	NT	+	−	−	−	+	−
	3	+	+	+	+	NT	NT	NT	+	+	−	−	+	−
	4	+	+	+	+	NT	NT	NT	+	−	+	+	+	−
	5	+	+	+	+	NT	NT	NT	+	−	−	+	+	+
	6	+	+	+	+	NT	NT	NT	+	+	−	+	+	−
	7	+	+	+	+	NT	NT	NT	+	+	+	+	+	+
HCV-related chronic active hepatitis	1	−	−	−	−	+	+	+	+	−	−	−	−	−
	2	+	+	+	−	+	+	+	−	−	+	+	+	−
	3	−	−	−	+	−	−	+	−	−	−	−	−	−
	4	−	−	+	−	−	−	+	−	+	−	+	+	−
	5	−	−	−	−	+	+	+	−	−	−	−	−	−
	6	−	−	+	+	−	+	−	−	+	−	+	+	−
	7	+	+	+	−	+	+	+	−	−	−	+	+	−
	8	−	−	−	−	+	−	−	−	−	−	−	+	−
	9	−	+	+	−	+	+	+	−	−	+	+	+	−
	10	−	−	+	−	−	+	−	−	−	−	−	−	−

C, HBV core region; X, HBV X region; S, HBV surface region; integration, HBV DNA integration; ccc, covalently closed circular HBV DNA; NT; not tested.

chronic active hepatitis, and HCV-related chronic active hepatitis in patients with low serum anti-HBc titers were 16, 100, and 25%, respectively. The number of integrated clones was small in fulminant hepatitis and acute hepatitis type B patients, but large in most HBV-related chronic active hepatitis patients, as demonstrated by the smeared bands. The integration of the HBV sequence is not necessary for persistent infection and clonal proliferation of hepatocytes is likely to occur following hepatocyte apoptosis and necrosis during the inflammatory phase. Thus, our results indicate that viral integration and clonal expansion of integrated cells may also occur during self-limited hepatitis.

Extrahepatic tissue tropism of HBV has been identified in mononuclear cells and various tissues [Dejean et al., 1984; Hosoda et al., 1990; Mason et al., 1993]. HBV DNA integration into the PBMC genome has been observed using Southern blotting, but the hybrid viral-cellular sequence was not reported [Catterall et al., 1994; Köck et al., 1996]. HBV integration was also demonstrated using a PCR-based method in the PBMCs of patients with HBsAg [Laskus et al., 1999]. Our results support these findings. Whether mononuclear cells support active HBV multiplication in vivo is a highly debated issue, and conflicting results have been obtained, particularly in regard to the detection of the covalently closed circular (ccc) DNA molecule, an intermediate in the synthesis of the viral pregenome and transcripts during productive HBV infection [Stoll-Becker et al., 1997]. Resolving this issue was not the main objective of the present study. Although we were unable to detect any ccc DNA molecules, the presence of a very small amount of free replicating HBV DNA forms could not be ruled out. The results mostly emphasize the persistence of integrated sequences.

The implications of the presence of such integrated viral sequences in mononuclear cells are unknown. The sensitivity limit of our assay implies that a positive result reflects the clonal proliferation of HBV DNA-integrated mononuclear cells. It remains to be established whether the viral integration reflects the prior infection of proliferating bone marrow cells or the stimulation of PBMC expansion, although the infection of bone marrow cells has been indeed reported [Romet-Lemonne et al., 1983]. In this study, we observed HBV integration in both liver tissue and PBMCs from HCV-related chronic active hepatitis patients positive for serum anti-HBc but negative for serum HBsAg.

In the present study, we identified the cellular genes adjacent to the integrated HBV DNA. In one acute hepatitis patient, the HBV DNA was integrated into the tumour necrosis factor (TNF)-induced protein, but this protein has never been characterized. TNF has a pivotal role in the organization and function of the immune system, and it has been implicated in the aetiology of several acquired and genetic diseases [Bodmer et al., 2002]. HBV DNA integration into cellular genes was also found in PBMCs. Moreover, some of the integrated viral genomes we detected in the liver and PBMCs were rearranged. Interestingly, rearranged HBV DNA was found in the liver of one patient with subacute fulminant hepatitis. Such rearranged HBV genomes have been described in numerous studies on patients with hepatocellular carcinoma (HCC) [Zhou et al., 1988; Meyer et al., 1992; Pineau et al., 1996] and in a few subjects with chronic hepatitis [Takada et al., 1990]. Thus, our data corroborate the view that the rearrangement of the HBV genome upon integration occurs is an early event during liver cell clonal expansion. The significance of these integrations in liver tissue and PBMCs in HBV-related/

HCV-related chronic active hepatitis patients is unknown. However, we have recently reported that some genes are affected by the character and expression of HBV DNA integration in HCC [Gozuacik et al., 2001]. Here, we show that HBV DNA integration is an early event in viral infection, suggesting that it may be one of the first genetic changes at to occur during tumorigenesis.

In approximately half of the patients with HCV-related chronic active hepatitis, HBV DNA was detected in the liver tissues and PBMCs using only one or two primer sets, suggesting the presence of integrated HBV DNA; HBV DNA integration was confirmed by Alu–PCR in the liver of 2 patients and in the PBMCs of two patients. In contrast to the results obtained for HBsAg-positive patients, only a few isolated bands were found after Alu–PCR amplification, suggesting a low viral copy number per cell, consistent with previous reports. In the remaining patients, the results were consistent with the presence of free HBV DNA molecules, but we could not exclude the existence of multiple HBV DNA integrations. No replicative intermediates of HBV were detected, except in case 1. Our study also detected only one or two regions of the HBV genome in the serum, livers and PBMCs of HBsAg-negative patients. Such patterns in the liver and PBMCs are consistent with the Alu–PCR data and previous investigations, showing a limited number of clones with deleted HBV genomes. Moreover, several investigators have reported that the sensitivity of the PCR protocol differs according to the sample type; for example, detection of the HBV S region was more sensitive in serum samples, whereas the detection of the X region was more sensitive in liver tissue samples [Jilg et al., 1995; Villa et al., 1995; Takeuchi et al., 1997; Koike et al., 1998]. With regards to the results obtained in the serum, prior investigations have shown transmissible and infectious HBV particles in the serum of anti-HBc- and anti-HBs-positive patients after the clearance of HBsAg and even in subjects negative for all HBV serological markers [Thiers et al., 1988]. While several studies have demonstrated the persistence of HBV DNA sequences in the serum, liver, and PBMCs of HBsAg-negative patients, our investigation provides the first evidence of the persistence of such viral genomes in an integrated form, confirmed by definite sequence determination. The impact of such persistent viral infections on the course of HBV/HCV co-infection and occult HBV infection has been underscored recently by several investigations in patients with chronic active hepatitis and HCC [Diamantis et al., 1994; Urashima et al., 1997; Tamori et al., 1999; Kawai et al., 2001].

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Characteristics of Patients with Chronic Hepatitis C who Develop Hepatocellular Carcinoma after a Sustained Response to Interferon Therapy

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BACKGROUND. The objective of the current study was to determine the characteristic features of sustained responders who develop hepatocellular carcinoma after treatment with interferon for chronic hepatitis C.

METHODS. This study included 3626 patients with chronic hepatitis C who had received interferon monotherapy. Cox proportional hazards analysis was used to compare sustained responders who did and did not develop hepatocellular carcinoma, and nonsustained responders who developed hepatocellular carcinoma in a multicenter, retrospective cohort study.

RESULTS. Among 1197 sustained responders, 27 patients developed hepatocellular carcinoma (2.3%). Compared with sustained responders who did not develop hepatocellular carcinoma, patients who developed disease more often were male ($P = 0.0212$), were older ($P = 0.0068$), and had advanced-stage histologic disease before interferon therapy ($P = 0.0345$). Conversely, compared with patients with hepatocellular carcinoma who were not sustained responders, patients who were sustained responders tended to be older at the time of the initiation of interferon therapy ($P = 0.0552$) and at the time hepatocellular carcinoma was detected ($P = 0.0593$), and they also were predominantly male ($P = 0.0507$). The histologic staging and serum aminotransferase levels at the initiation of interferon therapy, the interval to the detection of tumor, and the tumor size showed no significant differences between the two groups.

CONCLUSIONS. Sustained responders in the group at high risk for developing hepatocellular carcinoma after interferon therapy were older, more often were male, and had more advanced histologic disease stage. Such patients should be followed carefully periodically for > 10 years after they complete interferon therapy. *Cancer* **2004;101:1616–22.** © *2004 American Cancer Society.*

KEYWORDS: chronic hepatitis type C, hepatocellular carcinoma, interferon, sustained responder.

In Japan, chronic hepatitis C (CH-C) with advanced histologic staging often progresses to hepatocellular carcinoma (HCC),[1] although patients who are seropositive for antihepatitis C virus (anti-HCV) antibodies or for HCV RNA do not always progress to cirrhosis or HCC.[2,3] Risk factors for developing HCC in patients with CH-C are advanced histologic stage, irregular regeneration of hepatocytes, heavy drinking, higher serum alanine aminotransferase (ALT) levels or lower serum albumin levels, male gender, and older age.[1,4–7] Since 1992, patients with CH-C commonly have been treated with interferon α (IFN-α) or IFN-β, which are covered by public health insurance in Japan. Because IFN improves hepatic inflammation and inhibits the progression of hepatic fibrosis, it

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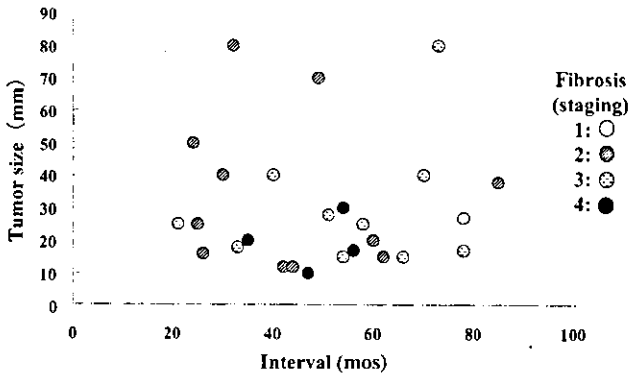

FIGURE 1. The interval from the completion of IFN therapy to the detection of SR HCC statistically did not correlate significantly with the tumor size or hepatic staging.

has been suggested that the incidence of HCC may be reduced by IFN treatment. In fact, IFN therapy reportedly was effective not only for improving liver biochemistry and eliminating HCV RNA but also for reducing the inflammation/fibrosis scores and lowering the risk of HCC, especially in sustained responders (SR patients).[8–14]

Although a significant decrease in the incidence of HCC has been observed in SR patients after IFN therapy,[9–14] HCC is detected in some of them.[15–25] The clinical features of SR patients who develop HCC (SR HCC patients) and the long-term incidence of HCC in SR patients remain unclear, and the optimal duration and frequency of follow-up have not been established. Therefore, we analyzed SR HCC patients to determine their characteristic features compared with SR patients who did not develop HCC (SR non-HCC patients) and non-SRs who developed HCC (non-SR HCC patients).

MATERIALS AND METHODS

Patients

For this study, 3626 patients with CH-C were enrolled (2344 males and 1282 females) who had received IFN therapy between January 1990 and November 2001. Data from these patients were collected from 6 institutions and related hospitals, including 1371 patients from Kyoto Prefectural University of Medicine, 1478 patients from Osaka University, 497 patients from Miyazaki Medical College, 130 patients from Nagoya University, 102 patients from Shinsyu University, and 48 patients from Yamaguchi University. All patients were seropositive for anti-HCV antibodies, positive for serum HCV RNA, and seronegative for hepatitis B virus surface antigen. We excluded patients who had coexisting liver diseases, such as autoimmune hepatitis or primary biliary cirrhosis, and confirmed that they did not abuse alcohol (daily alcohol intake > 60 g of ethanol). No patients were infected with human immunodeficiency virus (HIV). At the time of entry into this study, no patients showed evidence of HCC, as determined by ultrasonography (US) and/or computed tomography (CT) studies. In principle, patients underwent liver biopsy prior to IFN therapy, and the histologic diagnoses were reached according to the classification of Desmet et al.[26] The gender, mean age, and histologic disease stage at the initiation of IFN therapy are shown in Table 1.

TABLE 1
Characteristics of Patients with Chronic Hepatitis C who were Treated with Interferon[a]

Characteristic	Sustained responder	Nonsustained responder	*P* value[b]
No. patients	1197	2429	—
Male:female ratio	776:421	1568:861	0.8826
Age (yrs, mean ± SD)	49.4 ± 11.9	51.2 ± 10.6	< 0.0001
Histologic staging score: No. of patients (%)			
F1	385 (38.6)	522 (25.8)	
F2	322 (32.3)	613 (30.3)	< 0.0001
F3	262 (26.3)	782 (38.6)	
F4	29 (2.9)	109 (5.4)	
Not available	199	403	

SD: standard deviation; IFN: interferon.
[a] All data were determined before interferon therapy.
[b] *P* values were calculated with the Fisher exact probability test and the Wilcoxon two-sample test.

Natural IFN-α, recombinant IFN-α-2a, and recombinant IFN-α-2b were used in this study. In general, the IFN treatment protocol was within the range covered by public health insurance in Japan, namely, 3–10 MU of IFN-α for 24 weeks (daily for 2 weeks and 3 times per week for 22 weeks). In a few patients, administration of IFN-α was prolonged to 52 weeks. In some patients who suffered from severe side effects, the therapy period was shortened. In addition, patients for whom the total dose of IFN was < 200 MU were excluded from the study. Patients who had been treated with peginterferon or IFN/Ribavirin also were excluded. There was no difference noted with regard to the treatment protocol among the institutions and their related hospitals. We checked the laboratory findings at the end of IFN therapy and 6 months later. SR patients were defined as those who demonstrated continuous normal serum ALT levels for 6 months after finishing IFN therapy. The remaining patients were regarded as non-SR patients. The patient population included 1197 SR patients and 2429 non-SR patients.

We followed all patients for at least 1 year after the end of IFN therapy. The mean ± standard deviation

(SD) follow-up was 5.9 years ± 1.9 years. In SR patients, in general, we performed biochemical examinations, which sometimes included α-fetoprotein, every 3–12 months after confirming a sustained response. US and/or CT studies were performed at least once annually. However, because the incidence of HCC in non-SR patients—especially those with advanced-stage disease (fibrotic scores of F3 or F4)–was expected to be higher than that in SR patients, US and/or CT studies were performed every 3–6 months in non-SR patients. This strategy was similar in all of the institutions, and the frequency of radiographic examination was calculated to avoid unnecessary cost and not to miss HCC. However, some SR patients and non-SR patients who skipped or stopped visiting the outpatient clinic and some patients who were followed by their home physicians were not followed sufficiently. The diagnosis of HCC was based on appropriate radiologic findings (hepatic angiography, dynamic CT, magnetic resonance imaging).[27] When it was difficult to determine a final diagnosis with the radiologic findings, a histologic diagnosis was reached by tumor biopsy. In 17 of 27 SR HCC patients, a histologic diagnosis of HCC was obtained by the examination of resected hepatic tumors or biopsied tumor specimens. Patients who were diagnosed with HCC within 1 year after the end of IFN therapy were excluded from this study because of the possibility that a small but detectable HCC was missed before IFN therapy. Written informed consent to receive IFN therapy and to participate in this follow-up study was obtained from all patients, and the ethical committees of the participating institutions approved this study.

Statistical Analysis

Statistical analysis was performed using the SAS/PC statistical package (SAS Institute, Cary, NC). The Fischer exact probability test was used to compare the frequencies of gender. The Wilcoxon two-sample test was used to compare age, histologic staging, serum ALT level, interval between the end of IFN therapy and the detection of HCC, and the size of HCC. The independent risk factors for developing HCC in SR patients were examined by Cox proportional-hazards analysis; the variables were gender, age, histologic stage, and serum ALT level. Patients who had missing data were excluded from this analysis. Each variable was transformed into categorical data comprised of two-sample, ordinal numbers for multivariate analysis. P values were two-sided, and P values < 0.05 were considered statistically significant.

RESULTS

Characteristic Features of SR HCC Patients

During the observation of 3626 patients, HCC was detected in 259 patients; however, 19 patients were excluded, because HCC was detected within 1 year after they completed IFN therapy. The distribution of the remaining 240 HCC patients among the 6 institutions was as follows: 109 patients from Kyoto Prefectural University of Medicine (HCC incidence, 8.0%), 102 patients from Osaka University (HCC incidence, 6.9%), 3 patients from Miyazaki Medical College (HCC incidence, 0.6%), 15 patients from Nagoya University (HCC incidence, 11.5%), 8 patients from Shinsyu University (HCC incidence, 7.8%), and 3 patients from Yamaguchi University (HCC incidence, 6.3%). The incidence of HCC did not differ significantly among the institutions, except for Miyazaki Medical College. partly because hepatic fibrosis was less advanced in patients from this institution compared with patients from the other five institutions. Of 240 patients, 27 were SR patients, and 213 were non-SR patients. The ages of the 240 patients at the initiation of IFN therapy ranged from 37–77 years (mean age ± SD, 59.1 years ± 6.6 years) and varied from 39–83 years (63.6 years ± 6.8 years) at the time HCC was detected.

Among the 27 SR HCC patients, 5 patients consumed ≈ 50 g of ethanol daily. By evaluating liver specimens and biochemical examinations, including γ-glutamyl transferase, we excluded the possibility of alcoholic liver diseases in these patients. Serum HCV RNA was evaluated in the SR HCC patients by reverse transcriptase-polymerase chain reaction analysis. Twenty-six SR HCC patients were complete responders (seronegative for HCV RNA both at the end of IFN therapy and 6 months later), and 1 SR HCC patient was a biochemical responder (seropositive for HCV RNA at the end of IFN therapy). In 1 complete responder who developed HCC, serum HCV RNA became positive 12 months after completing IFN therapy.

No correlation could be found between the interval before HCC was detected, tumor size, or hepatic histologic stage among the SR HCC patients (Fig. 1). HCC that was detected long after discontinuing IFN therapy was not always large, and the patients with large HCC did not always show more advanced stage according to liver histology. The greatest dimensions of the 2 largest SR HCC tumors were 80 mm and were detected 32 months and 73 months after the end of IFN therapy. The greatest dimension of SR HCC found after the longest interval (85 months) was 38 mm.

Tumor tissue samples could be examined from 18 of 27 SR HCC patients. Two samples were categorized

TABLE 2
Comparisons between Sustained Responders with and without Hepatocellular Carcinoma[a]

Characteristic	SR HCC	SR non-HCC	*P* value[b]
No. of patients	27	1170	
Male:female ratio	25:2	751:419	0.0016
Age (yrs, mean ± SD)	60.7 ± 7.5	50.2 ± 12.4	< 0.0001
Serum ALT (IU/L, mean ± SD)	111.7 ± 67.7	122.6 ± 109.9	0.7267
Histologic staging score: No. of patients (%)			
F1	1 (3.7)	384 (39.6)	
F2	11 (40.7)	310 (32.0)	< 0.0001
F3	10 (37.0)	252 (26.0)	
F4	5 (18.5)	24 (2.5)	

SR: sustained responder; HCC: hepatocellular carcinoma; SD: standard deviation; ALT: alanine aminotransferase; IFN: interferon.

[a] All data were determined before interferon therapy.

[b] *P* values were calculated with the Fisher exact probability test and the Wilcoxon two-sample test.

TABLE 3
Factors Associated with the Development of Hepatocellular Carcinoma in Sustained Responders[a]

Characteristic	Risk ratio	95% CI	*P* value
Male vs. female	5.498	1.290–23.439	0.0212
Age	7.378	1.737–31.326	0.0068
Stage of liver disease	2.344	1.064–5.164	0.0345
Serum ALT	1.331	0.606–2.923	0.4768

95% CI: 95% confidence interval; ALT: alanine aminotransferase.

[a] All data were determined before interferon therapy. Statistical analysis was performed using the Cox proportional hazards test. The variable for age was set at < 50 years or ≥ 50 years, the variable for stage was set < F3 or ≥ F3, and the variable for the serum alanine aminotransferase level was set at < 88 UI/L or ≥ 88 UI/L. The variables age and serum alanine aminotransferase level were determined as median data. The variable for stage was set to obtain the largest hazard ratio.

as well differentiated HCC, 11 samples were moderately differentiated HCC, 2 samples were poorly differentiated HCC, and 2 samples were undifferentiated HCC. One sample was the necrotic tissue after transcatheter arterial embolization therapy (TAE). Nontumorous liver tissue samples from 18 patients were evaluated for their fibrosis scores in resected HCC or tumor biopsy specimens. Liver fibrosis scores improved in nine patients, did not change significantly in eight patients, and worsened in one patient.

Sixteen of 27 SR HCC patients underwent partial hepatectomy, and 10 patients were treated with TAE and/or percutaneous ethanol injection therapy. Because one patient changed his hospital after the diagnosis of HCC, we could not know his prognosis.

Comparison between SR HCC Patients and SR Non-HCC Patients

We compared 27 SR HCC patients with 1170 SR non-HCC patients. The SR HCC patients included 25 males (92.6%) and 2 females (7.4%), and the SR non-HCC patients included 751 males (63.5%) and 419 females (35.8%). At the time IFN therapy was initiated, the mean age of the SR HCC patients was 60.7 years ± 7.5 years (range, 37–70 years), whereas the mean age of the SR non-HCC patients was 50.2 years ± 12.4 years (range, 17–73 years). Thus, the SR HCC patients more often were male ($P = 0.0016$) and were older ($P < 0.0001$) compared with the SR non-HCC patients (Table 2).

The fibrotic scores in biopsied liver specimens before IFN therapy for the SR HCC patients included 1 F1 specimen (3.7%), 11 F2 specimens (40.7%), 10 F3 specimens (37.0%), and 5 F4 specimens (18.5%); and the fibrotic scores for the SR non-HCC patients included 384 F1 specimens (39.6%), 310 F2 specimens (32.0%), 252 F3 specimens (26.0%), and 24 F4 specimens (2.5%). The 2 female SR HCC patients both had F4 specimens. Among the total SR population, SR HCC patients had more advanced-stage disease ($P < 0.0001$). The mean serum ALT level at the initiation of IFN therapy was 111.7 IU/L ± 67.7 IU/L in the SR HCC patients and 122.6 IU/L ± 109.9 IU/L in the SR non-HCC patients (Table 2).

Cox proportional-hazards analysis of factors associated with the development of HCC in the SR patients was performed with four variables (gender, age, histologic stage, and serum ALT level). In this analysis, the hazard ratios for age, stage, and serum ALT level were calculated between the two groups. The age variable was set at < 50 years or ≥ 50 years, the fibrotic score (stage) variable was set at < F3 or ≥ F3, and the variable for serum ALT level was set at < 88 IU/L or ≥ 88 IU/L. The variables age and serum ALT level were determined as median data. We chose the variable for stage to obtain the greatest hazard ratio. The SR HCC patients more often were male ($P = 0.0212$, 95%CI, 1.290–23.439), were older ($P = 0.0098$, 95%CI, 1.737–31.326), and had advanced-stage disease according to liver histology ($P = 0.0345$; 95%CI, 1.064–5.164) before IFN therapy. Gender, age, and histologic stage before IFN therapy were considered independent risk factors for the development of HCC (Table 3).

Comparison between SR HCC Patients and Non-SR HCC Patients

We compared the clinical characteristics of the 27 SR HCC patients with the 213 non-SR HCC patients. The non-SR HCC patients included 161 males (75.6%) and 52 females (24.4%). The mean age of the non-SR HCC patients at the initiation of IFN therapy was 58.9 years ± 6.5 years (range, 40–77 years), and the mean age at

TABLE 4
Comparisons between Sustained Responders and Nonsustained Responders among Patients with Hepatocellular Carcinoma

Characteristic	SR	Non-SR	*P* value[a]
No. of patients	27	213	
Male:female ratio	25:2	161:52	0.0507
Age at the initiation of IFN (yrs, mean ± SD)	60.7 ± 7.5	58.9 ± 6.5	0.0552
Age at the detection of HCC (yrs, mean ± SD)	65.1 ± 7.8	63.4 ± 6.7	0.0593
Serum ALT (IU/L)[b]	111.7 ± 67.7	120.5 ± 56.4	0.2027
Histologic staging score: No. of patients (%)[b]			
F1	1 (3.7)	12 (5.6)	
F2	11 (40.7)	36 (16.9)	0.1861
F3	10 (37.0)	135 (63.4)	
F4	5 (18.5)	30 (14.1)	
Interval (mos, mean ± SD)[c]	49.3 ± 18.2	49.7 ± 24.8	0.7484
Tumor size (mm, mean ± SD)	31.2 ± 20.1	21.3 ± 9.9	0.1573

SR: sustained responder; IFN: interferon; SD: standard deviation; HCC: hepatocellular carcinoma; ALT: alanine aminotransferase.

[a] *P* values were calculated with the Fisher exact probability test and the Wilcoxon two-sample test.

[b] Data were determined before interferon therapy.

[c] The interval was between the completion of interferon therapy and the detection of hepatocellular carcinoma.

time HCC was detected was 63.2 years ± 6.7 years (range, 44–83 years). The mean serum ALT level in the non-SR HCC patients at the start of IFN therapy was 120.5 IU/L ± 56.4 IU/L. The fibrotic scores of biopsied liver specimens obtained from the non-SR HCC patients before IFN therapy included 12 F1 specimens (5.6%), 36 F2 specimens (16.9%), 135 F3 specimens (63.4%), and 30 F4 specimens (14.1%). Thus, concerning gender and age, the SR HCC patients tended to be predominantly male ($P = 0.0507$) and were older (both at the initiation of IFN therapy [$P = 0.0552$] and at the time HCC was detected [$P = 0.0593$]) compared with the non-SR HCC patients; however, the serum ALT levels and the histologic stage before IFN therapy among the SR HCC patients did not differ significantly compared with the non-SR HCC patients (Table 4).

The mean interval between the end of IFN therapy and the detection of HCC for the SR HCC patients was 49.3 months ± 18.2 months (range, 21–85 months), which was not significantly different from that for the non-SR HCC patients (49.7 months ± 24.8 months; range, 12–141 months). The mean greatest dimension of SR HCC was 31.2 mm ± 20.1 mm, which was slightly greater than, but not significantly different from, the mean greatest dimension of non-SR HCC (21.3 mm ± 9.9 mm) (Table 4).

DISCUSSION

In the current study, we compared the clinical characteristics of SR HCC patients with the characteristics of SR non-HCC patients to determine the characteristic features of SR HCC. The incidence of HCC among the 1197 SR patients was 2.3%, and the incidence among the 2429 non-SR patients was 8.8% during the mean follow-up of 5.9 years. In patients with CH-C, aging and advanced hepatic histologic stage reportedly are major risk factors for HCC development.[1,4] This was true for the SR population in our current investigation, because the risk ratio for developing HCC was > 7 times greater in older patients (≥ 50 years) and was more than twice as high in patients who had advanced histologic stage disease (fibrotic score ≥ F3) according to a Cox proportional-hazards analysis. Khan et al. also reported that male gender is an important risk factor for HCC development.[5] In the current study, males were more than five times more likely to develop HCC in the SR population. Thus, older male patients with advanced hepatic fibrosis were considered to be a high-risk group for developing HCC among the SR population (Table 3).

Conversely, compared with the non-SR HCC patients, the SR HCC patients were older at the initiation of IFN therapy ($P = 0.0552$) and at the detection of HCC ($P = 0.0593$), and they were predominantly male ($P = 0.0507$). Although these characteristics may not have differed significantly in the current study, a study of even larger size may show that this indeed is a trend. The histologic staging, the serum ALT level at the initiation of IFN therapy, the interval for the detection of HCC, and the tumor size did not differ significantly between the two groups. The tumor size in SR HCC patients was slightly greater compared with the tumor size in non-SR HCC patients, most likely because of the extended interval of screening for HCC after patients attained a sustained response to IFN therapy (Table 4).

Some previous articles reported that HCV RNA may survive in the hepatic tissues of SR HCC patients[28–30] and may be involved in the carcinogenesis or growth of HCC. Although we could not demonstrate the presence of HCV RNA in tumors and surrounding hepatic tissues from SR HCC patients, eradication of HCV from these tissues, along with the nontumorous hepatic tissues, was confirmed in several previous studies,[15–21] suggesting that the persistence of HCV is not essential for the growth of HCC in SR patients.

To ascertain the time of HCC occurrence, several studies were performed that examined the doubling time (DT) of HCC. Two studies from Japan reported that the DT of HCC measuring < 3 cm in greatest dimension was 93.0 days ± 57.4 days or 195.0 days ± 171.0 days.[31,32] Barbara et al. reported that the DT of HCC measuring < 5 cm in greatest dimension was 204.2 days ± 135 days.[33] Recently, Toyoda et al. re-

ported similar results, assuming that the greatest dimension of occult HCC was 5 mm before IFN therapy.[34] We calculated the growth interval between a single HCC cell and an HCC measuring 1 cm in greatest dimension on the assumption that the DT of HCC was 90 days and concluded that the growth interval may be > 6 years.[8] Because smaller and well differentiated HCCs have a longer DT, the growth interval to reach 1 cm in greatest dimension may be much longer than 6 years. Therefore, it is probable that small HCC may have existed in the liver prior to IFN therapy in the current SR HCC patients.[35]

It cannot be determined with certainty how long SR patients should be followed after they complete IFN therapy. Judging from the results obtained in the current study, we recommend that, when SR patients are male, age > 50 years old, and have F3 or F4 histologic stage, they should be checked by US or CT at least twice per year for > 10 years. Other SR patients with less advanced disease should be checked at least once per year.

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Practice of Interferon Therapy
—Chronic hepatitis C (combination with ribavirin)—

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Abstract: Interferon (IFN) plus ribavirin therapy over 24 weeks resulted in a 20% complete response (CR) in chronic hepatitis C (CH-C) patients who had relapsed on IFN monotherapy in Japan. As we reported previously, the serum amount of HCV RNA decreases biphasically during IFN therapy in CH-C patients. It has been considered that the first phase reflects a direct antiviral effect of IFN and the second phase might reflect antiviral activity and elimination of hepatocytes by apoptosis which might be induced by activated CTL. The second phase might be an important factor in achieving a complete response (CR) in antiviral therapy. Serum HCV RNA level, HCV genotype, amino acid changes in NS5A region, and stage of liver fibrosis are important predictive factors in IFN monotherapy in CH-C patients. However, these factors were not so useful for predicting CR in IFN/ribavirin therapy in the Japanese study. To clarify the predictive factors on IFN/ribavirin in CH-C patients, we are going to study HCV dynamics, changes of Th1/Th2 balance in peripheral blood, and changes of receptors of Th1 and Th2 in peripheral blood on IFN/ribavirin therapy in CH-C patients.

Key words: Chronic hepatitis C; Interferon; Ribavirin; HCV dynamics; First phase; Second phase

Introduction

In Japan, there have been hundreds of thousands of patients with intractable chronic hepatitis C who have not responded to interferon (IFN) therapy in Japan. The combination of IFN/ribavirin, which became covered by the Japanese medical insurance in December 2001, can treat around 20% of such patients. Although only the data from clinical studies are available for the follow-up results for many patients, this paper describes the indications, therapeutic outcomes, and adverse reactions of the combination therapy. The combination

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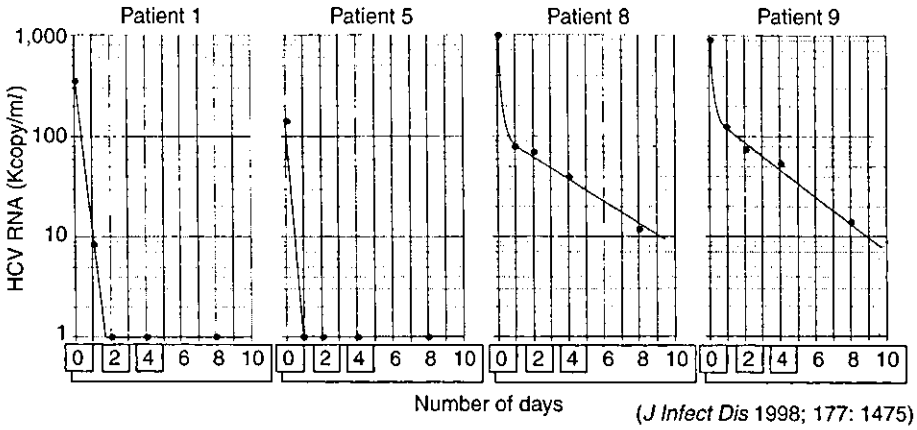

Fig. 1 Blood HCV RNA dynamics during IFN therapy

therapy has been used widely over the past few years in Western countries. The combination of peginterferon (Peg-IFN), a long-acting preparation of IFN, and ribavirin, is now the standard therapy for intractable hepatitis C.

Characteristics of IFN/Ribavirin Combination Therapy

IFN has both antiviral and immunomodulating effects. Ribavirin (Rib) is a nucleic acid derivative with a relatively weak antiviral effect. It mainly acts on the immune system: it inhibits the Th2 system to make the Th1 system relatively superior, and it directly stimulates the Th1 system.[1-3] The IFN/Rib combination therapy produces a significantly higher response rate than the IFN therapy alone in patients with chronic hepatitis C.

We previously examined the blood HCV dynamics after the consecutive administration of IFN in patients with chronic hepatitis C, and reported that the serum amount of virus (amount of HCV RNA) was reduced in a biphasic manner[4]: the serum amount of virus was acutely reduced in the first 24 hours (1st phase, half life: 5 to 7 hours) and then slowly in the subsequent period (2nd phase, half life: 70 to 100 hours) (Fig. 1).[4]

The HCV dynamics after the administration of IFN can be roughly divided into 4 patterns: (1) the HCV RNA amount is sharply reduced to 10 to 1% (or lower) of that of the pretreatment level, and comes close to or falls below the minimum limit of determination in the 1st phase; (2) the HCV RNA amount is reduced to around 10% of that of the pretreatment level in the 1st phase, with a subsequent half life that is 5 to 10 times as long as that of the 1st phase; (3) the HCV RNA amount is significantly reduced in the 1st phase, followed by poor reduction during the second phase, with HCV RNA remaining positive even after several months; and (4) no significant viral reduction during either the 1st or 2nd phase.

It is considered that the 1st phase represents the direct antiviral effect of IFN, while the 2nd phase represents the antiviral effect plus the removal of infected hepatocytes by cytotoxic T lymphocytes (CTL).[5] Hepatitis C with the above (3) or (4) HCV dynamics pattern cannot be eliminated with IFN therapy. It is assumed that the IFN/Rib combination may increase the antiviral effect of IFN and stimulate CTL to remove infected hepatocytes and reduce the half-life of the 2nd phase, resulting in an increase in the rate of marked response. In fact, clinical results supporting this assumption have been reported.[6,7] It is possible that some of the patients who showed the above (2) or (3) pattern after IFN therapy may well respond to the IFN/Rib combination.

Indications

Basically, the indications of the IFN/Rib combination are the same as those of IFN therapy. However, it should be noted that Rib dose-dependently causes hemolytic anemia, and that it is contraindicated in patients with a pretreatment hemoglobin level of 12 g/dL or lower because it reduces hemoglobin by 3 to 4 g/dL, on an average. Other adverse reactions to the combination therapy are similar to those of IFN therapy. However, general malaise, anorexia, alopecia, and eruptions tend to be slightly severer with the combination therapy. Therefore, it is generally contraindicated in patients of 70 years or older, and should be carefully administered in patients of 65 years or older.

Table 1 Therapeutic Efficacy of IFN in 1,370 Patients with Chronic Hepatitis C

Disease stage	Therapeutic efficacy of IFN (biochemical efficacy determination)		
	Marked response	Transient response	No response
F1 (n=229)	101 (44%)	76 (33%)	52 (23%)
F2 (n=710)	236 (33%)	195 (27%)	279 (40%)
F3 (n=383)	85 (22%)	80 (21%)	218 (57%)
F4 (n= 48)	4 (8%)	7 (15%)	37 (77%)
Total 1,370	426 (31%)	358 (26%)	586 (43%)

(Okanoue, T. *et al.*: *Hepatol Res* 2002)

Therapeutic Practice and results

Important predictive factors for the therapeutic efficacy of IFN for chronic hepatitis C include serum viral amount, HCV genotype, and severity of hepatic fibrosis. Table 1 shows the efficacy of IFN in 1,370 patients with hepatitis C by the disease stages (severity of fibrosis). It shows that 31% of the patients markedly responded to IFN.[8] It should be noted that since 10% of the 31% still had viremia, they are so-called biochemically marked responders.

The Japanese clinical study for developing the IFN/Rib combination therapy was performed in intractable cases with a genotype of 1b and HCV RNA amount of 1 Meq/mL or higher. The following 3 groups were set in the study: a group treated with IFN at 6 MU/day (6 MU/day for 2 weeks consecutively followed by thrice weekly) and Rib; a group treated with IFN at 10 MU/day (10 MU/day for 2 weeks consecutively followed by thrice weekly at 6 MU/day) and Rib, and a group treated with IFN alone. All three groups were treated for a total of 24 weeks. The rate of marked responders was 2.3% in the IFN group and around 20% in the IFN 6 MU/day + Rib combination group (Table 2). Rib was administered at a dose of 600 mg/day in patients weighing 60 kg or less and 800 mg/day in those weighing 61 kg or more. The patients who entered the study were intractable cases, and about three quarters had been treated with IFN without sufficient re-

Table 2 IFN/Rib Combination Therapy for Intractable Chronic Hepatitis C (comparison with IFN therapy)

Therapeutic contents	Marked response rate
rIFNα-2b, 6 MU for 2 weeks continuously, followed by 6 MU thrice weekly for 22 weeks plus ribavirin	20.2% (18/89)
rIFNα-2b, 10 MU for 2 weeks continuously, followed by 6 MU thrice weekly for 22 weeks plus ribavirin	17.0% (16/92)
rIFNα-2b, 10 MU for 2 weeks continuously, followed by 6 MU thrice weekly for 22 weeks (IFN alone)	2.3% (2/88)

Patients with a gene type of 1b and large amounts of virus in the blood were treated, and about 75% of them had shown no response to IFN alone.

sults. About 50% of them were very intractable with an HCV RNA amount of 850 KIU/mL or higher. The results obtained were not so different from those obtained in Western countries. In Western countries, IFN/Rib combination therapy is given for 48 weeks in intractable chronic hepatitis C patients,[9] and the combination of Rib and Peg-IFN, a long-acting preparation of IFN, has recently become the first line therapy for intractable chronic hepatitis C.[10,11]

Since the rate of marked response in the IFN/Rib combination therapy is proportional to the blood Rib concentration, Rib should be administered at 1,000 mg/day in patients weighing 75 kg or more.

Predictive Factors of Efficacy

Serum viral amount, genotype, and severity of hepatic fibrosis are important factors that influence the therapeutic efficacy of IFN therapy. The rate of marked response by IFN is almost 0% in patients with a serum viral amount of 850 KIU/mL or more. In contrast, the IFN/Rib combination therapy in patients with a genotype of 1b and a large serum viral amount showed a marked response rate of 19.2% in the group treated with 6 MU/day of INF even in patients with a serum viral amount of 850 KIU/mL or more. This indicates a certain rate of marked response can be expected from the combination therapy in patients with a high viral amount. Although the marked response rate of IFN therapy is significantly reduced as hepatic fibrosis progresses, it is not so reduced as the IFN therapy in patients treated with the combination therapy (F1: 23%, F2: 18%, and F3: 15%). Therefore, it is impossible to accurately predict the efficacy of the IFN/Rib combination therapy, and it is worth trying it in any patients for whom it is indicated.

The presence/absence of blood HCV RNA at 4 or 12 weeks after the start of treatment is an important predictor for the efficacy of the ongoing combination therapy: 56% of those negative for blood HCV RNA determination at 4 weeks recovered completely, and 27% of those positive at 4 weeks but negative at 12 weeks showed marked response. In contrast, there is little possibility of recovery for those positive for HCV RNA at 12 weeks.

We are now examining the relationship between HCV dynamics and Th1/Th2 balance or the expression of Th1 and Th2 cytokine receptors to identify a predictive factor of the efficacy of the IFN/Rib therapy. It is considered important to stimulate the Th1 system by Rib and increase the antiviral effect of IFN by the combination in order to treat patients not responding to IFN therapy with the IFN/Rib combination therapy.

Adverse Reactions

As described above, Rib surely causes hemolytic anemia. Rib should be reduced when Hb falls to 10 g/dL, and returned to the original level when Hb increases. Anemia is most likely to occur 2 to 4 weeks after the start of treatment, although it may progress after 4 weeks.

The IFN/Rib combination therapy should be given carefully to patients with hypertension or diabetes, particularly those with a change of the fundus occuli, because they may develop cerebral hemorrhage during treatment.

Conclusions

IFN-Rib combination therapy is the first line therapy for intractable chronic hepatitis C. However, because much remains to be improved (such as lessening the relatively severe adverse reactions), it should be applied carefully.

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