ABI PRISM Genetic Analyzer 310 (Applied Biosystems). To verify whether the polymorphism found affected the amplification efficiency of real-time PCR for measuring PVLs for the *pol* region, PCR products were subcloned by pGEM-T Easy vector system (Promega, Madison, WI). The amplification efficiency of real-time PCR for the *pol* region was compared between the DNA sequences with and without this polymorphism.

Detection of Type 1 defective provirus by long PCR

To assess whether the Type 1 defective provirus exists in the HTLV-1 carriers with lower PVLs for the *pol* region compared to those for the *pX* region, long PCR, which amplifies the complete provirus and the Type 1 defective provirus with 5′ LTR conserved, was performed. The primers were as follows: 5′LTR(HTLV-0647F 5′-GTTCCACCCCTTTCCCTTTC ATTCACGACTGACTGC-3′: positions 647–682) and 3′LTR (HTLV-8345R 5′-GGCTCTAAGCCCCCGGGGGATATTTG GGGCTCATGG-3′: positions 8345–8319).[18] Long PCR was performed using LA Taq DNA polymerase (Takara Bio, Shiga, Japan). Cycles for long PCR were as follows: one cycle of 98°C for 20 sec, 35 cycles of denaturation at 98°C for 10 sec, annealing at 65°C for 20 sec and extension at 72°C for 7 min. Genomic DNA containing 100 copies of HTLV-1 provirus for the *pX* region from each subject was used. To ensure that same amount of provirus was used for each reaction, PCR for the *pX* region was performed as an internal control. Primers used for this PCR were as follows: the forward primer (HTLV-7396F 5′-GGCGACTGGTGCCCCATCT CTGGGGGACTATGTTCG-3′: positions 7396–7431) and the reverse primer described above (HTLV-8345R).

DNA sequence analysis for Type 1 defective provirus

Long PCR products from subjects suspected of having defective provirus were subcloned by pGEM-T Easy vector system (Promega). The resulting plasmid DNA was purified by GenElute Plasmid Miniprep Kit (Sigma-Aldrich, St. Louis, MO). The DNA sequence of long PCR product was identified using an ABI PRISM Genetic Analyzer 310 (Applied Biosystems).

Southern blot hybridization analysis

To analyze the clonality of HTLV-1-infected cells, Southern blot analysis for HTLV-1 provirus was performed based on the method previously described by Kamihira *et al.* with slight modification.[17] Genomic DNA samples (10 μg) from cases were digested with restriction enzyme *Eco*RI (Fermentas, Barlington, Canada), electrophoresed on 0.7% agarose gel and transferred to nylon membrane (Roche). The filter was hybridized with DIG-PCR-labeled HTLV-1 DNA probe mix, which was prepared by a mixture of PCR products to cover the genome of 5′LTR-*gag* (positions 655–1624), *pro* (positions 2109–2619), *pol* (positions 3410–4059), *env* (positions 5464–6114) and *pX* (positions 7461–8646) and by incorporating DIG-11-dUTP (Roche). Finally, the band patterns were visualized with a CDP-star (Roche).

Results

PVLs of 208 asymptomatic HTLV-1 carriers based on the real-time PCR for the pol and pX regions

PVLs of 208 asymptomatic HTLV-1 carriers were determined by real-time PCR using primers for the *pol* and *pX* regions. The mean PVLs determined using primers for the *pol* region (2.3 copies per 100 PBMCs) were lower than that for the *pX* region (3.6 copies per 100 PBMCs). Because the *pX* region has been reported to be conserved in the HTLV-1 provirus,[14,16] the carriers, whose PVLs for the *pol* region were much lower than those for the *pX* region, were assumed to have many PBMCs harboring defective HTLV-1 provirus. Therefore, to characterize the carriers with defective HTLV-1 provirus, the subjects with relatively high PVLs for the *pX* region, which were equal to or greater than 1.0 copy per 100 PBMCs, and with PVLs for the *pol* region, which were less than half of those for the *pX* region, were supplied for further analysis. Seven carriers (Cases A–G) among 111 carriers with PVLs for the *pX* region, which were equal to or greater than 1.0 copy per 100 PBMCs, met this condition (Table 1).

DNA polymorphism analysis for the pol primer region

Although these seven carriers were potential carriers with relatively high PVLs and defective provirus, there was a possibility that the low PVLs for the *pol* region were due to the polymorphism of the DNA sequence of primers and probe for the *pol* region. Therefore, DNA sequences of the *pol* regions for PCR in Cases A–G were determined by the direct sequencing of PCR products. In Cases A–G, the polymorphism was not detected in the forward primer and probe annealing sequences. However, as shown in Table 1, the polymorphism of the DNA sequence was identified in two positions (3860 A>C and 3876 G>A) of the genome of provirus for the reverse primer for the *pol* region in four of seven cases (Cases D–G, Table 1). This DNA sequence was cloned into the plasmid, and the amplification efficacy of real-time PCR for the *pol* region was assessed. As expected, the amplification efficacy of real-time PCR in the plasmid with two nucleotide substitutions was ~3–4% of that in the plasmid without nucleotide substitutions (data not shown). These results accounted for the low PVLs for the *pol* region in Cases D–G shown in Table 1. Therefore, only Cases A–C were thought to potentially have many PBMCs with defective HTLV-1 provirus.

Sequential change of PVLs determined by real-time PCR for the pol and pX regions

All of three cases (Cases A, B and C) were followed-up for 10 or more years, and the samples from several screens were available (Fig. 2). None of these cases showed any signs or

Table 1. Proviral DNA loads for the *pol* and *pX* regions and polymorphism found in the *pol* region

Cases	Sex	Age (years)	PVLs (copies/100 PBMCs)			Polymorphism of *pol* region	
			pX	*pol*	*pX/pol*	3860[1]	3876[1]
A	Male	61	57.5	2.8	21.7	A	G
B	Female	73	31.7	0.5	59.2	A	G
C	Female	84	17.3	3.8	4.6	A	G
D	Male	82	12.6	0.4	5.5	C	A
E	Male	45	3.7	0.1	30.6	C	A
F	Male	75	2.5	0.1	27.2	C	A
G	Male	83	1.9	0.1	24.5	C	A

PVLs: proviral DNA loads; PBMCs: peripheral blood mononuclear cells.
[1]Position of proviral genome sequence.

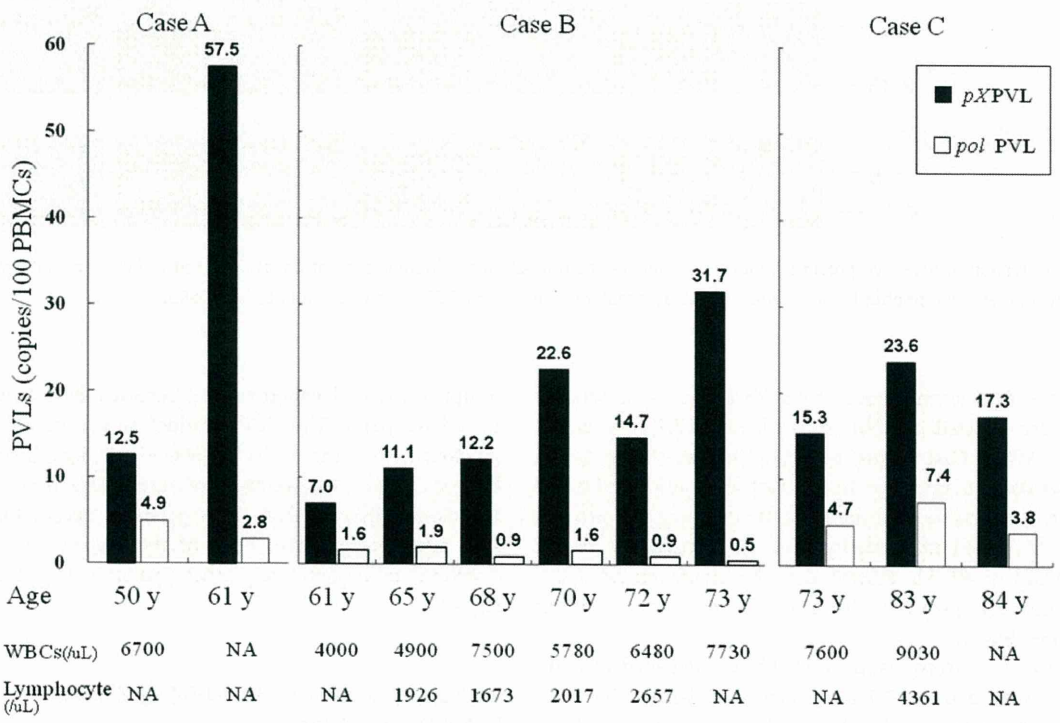

Figure 2. Proviral DNA loads for *pol* and *pX* regions at different ages of Cases A, B and C. WBCs: white blood cells; NA: not available.

symptoms suggesting ATL- and HTLV-1-associated diseases. The numbers of white blood cells and lymphocytes in their peripheral blood were within normal limits with no abnormal cells observed during the follow-up period. Cases A, B and C had high PVLs for the *pX* region, which were greater than 15 copies per 100 PBMCs at the most recent screens, when the cases were 61, 73 and 84 years old, respectively. PVLs for both the *pol* and *pX* regions were measured in previous time-sequential samples from these cases (Fig. 2). PVLs for the *pX* region in Cases A and B showed a marked increase during the 11- and 12-year follow-up, and those for the *pol* region showed either no change or decreased.

Sequencing and analysis for defective provirus in three cases

Long PCR to amplify the HTLV-1 provirus using primers for 5′ LTR and for the *pX* region was performed in the time-sequential samples from Cases A, B and C (Fig. 3). This long PCR amplifies the complete provirus and the Type 1 defective provirus with 5′ LTR conserved. In other words, Type 2 defective provirus, which does not conserve 5′LTR, is not amplified by this long PCR. If the subject had a complete proviral genome, the size of PCR product would be expected to be 7.7 kb. If the PCR products were smaller than 7.7 kb, they were judged to be derived from Type 1 defective provirus.

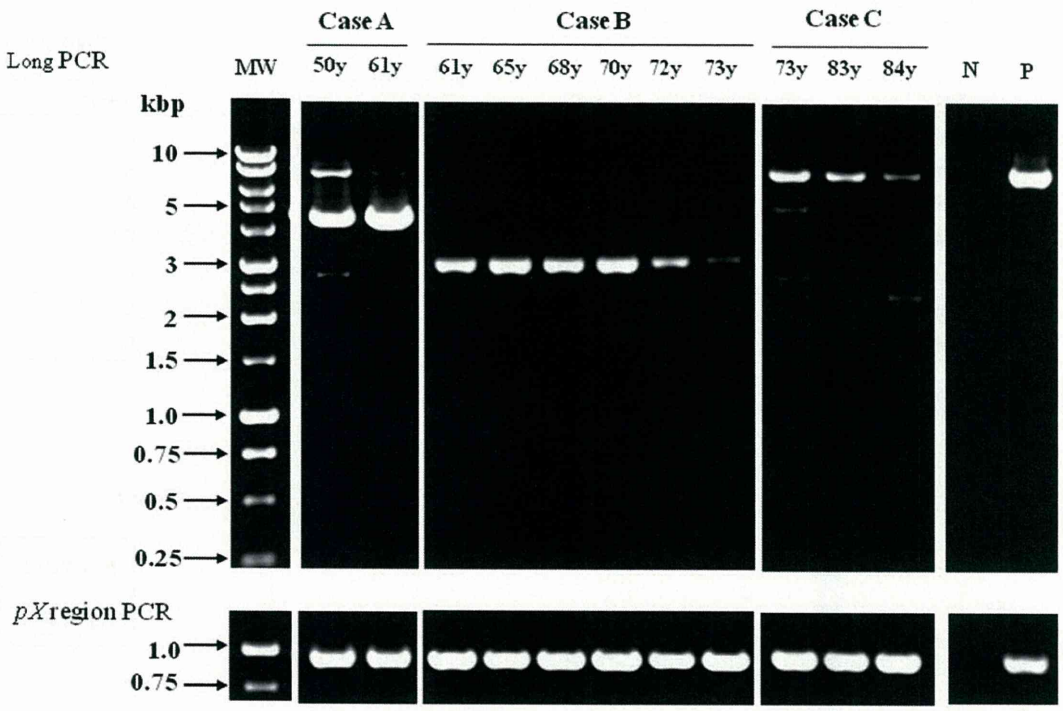

Figure 3. Detection of defective provirus by long polymerase chain reaction at different ages of Cases A, B and C. MW: molecular weight marker; N: human T-lymphotropic virus Type 1 (HTLV-1) negative subject; P: HTLV-1-positive cell line, ED-40515.

In Case A, a strong band of 4.5 kb and a weak band of 2.7 kb were detected in addition to a band of 7.7 kb at age of 50 years. When Case A was 61 years old, the strong 4.5 kb band increased its intensity. In contrast, the weak band of 2.7 kb was not detectable, and the band for the complete proviral genome decreased its intensity. The DNA sequence of the strong band of 4.5 kb showed that this band represented a Type 1 defective provirus with a 3.2-kb deficiency (positions 1203–4368, Fig. 4).

In Case B, a strong band of 2.9 kb was detected in addition to a weak band of 7.7 kb at age of 61 years. The DNA sequence of this 2.9 kb band showed that this band also represented a Type 1 defective provirus with 4.8 kb deficiency (positions 1173–5958, Fig. 4). When Case B was 73 years old, the intensity of the 2.9 kb band decreased markedly (Fig. 3). However, PVLs in Case B gradually increased as time passed (Fig. 2). Therefore, HTLV-1-infected cells harboring 2.9-kb Type 1 defective provirus were assumed not to be responsible for the increase of PVLs in Case B. In other words, HTLV-1-infected cells harboring provirus, which was not detected by long PCR used in our study, increased in number.

In Case C, several bands smaller in size than 7.7 kb, which might represent different Type 1 defective provirus, were detected. However, they were not consistently detectable at the ages of 73, 83 and 84. The 7.7-kb band of the complete proviral genome also decreased its intensity at the age of 84 years. The PCR product at age of 83 years was subcloned, and the DNA sequence was identified (Fig. 4). Thirteen Type 1 defective proviruses were detected in the 33 colonies derived from PCR products except for provirus with complete genome. Four of these were found to have insertions of nonviral sequences (clone cc-1,-3,-4 and -6, in Fig. 4).

Analysis of clonality of HTLV-1-infected cells by Southern blotting

To examine the clonal expansion of HTLV-1-infected cells, samples of genomic DNA (10 μg) from Cases A, B and C were analyzed by Southern blotting (Fig. 5). In Case A, a 17-kb band (a-1) was detected both at 50 and 61 years of age. The intensity of a-1 increased markedly at age 61. The increased intensity of clone a-1was consistent with the finding of increased PVLs for the *pX* region (Fig. 2) and with the increased intensity of the 4.5-kb band of Type 1 defective provirus by long PCR (Fig. 3). In addition, another weak band (a-2) was detected at age 61. Because the size of a-2 was ~7 kb, which was smaller than the size of complete HTLV-1 provirus (9 kb), a-2 was considered to be a clone with defective provirus, which was not detected by long PCR. In Case B, two clones (b-1 and b-2) were detected both at

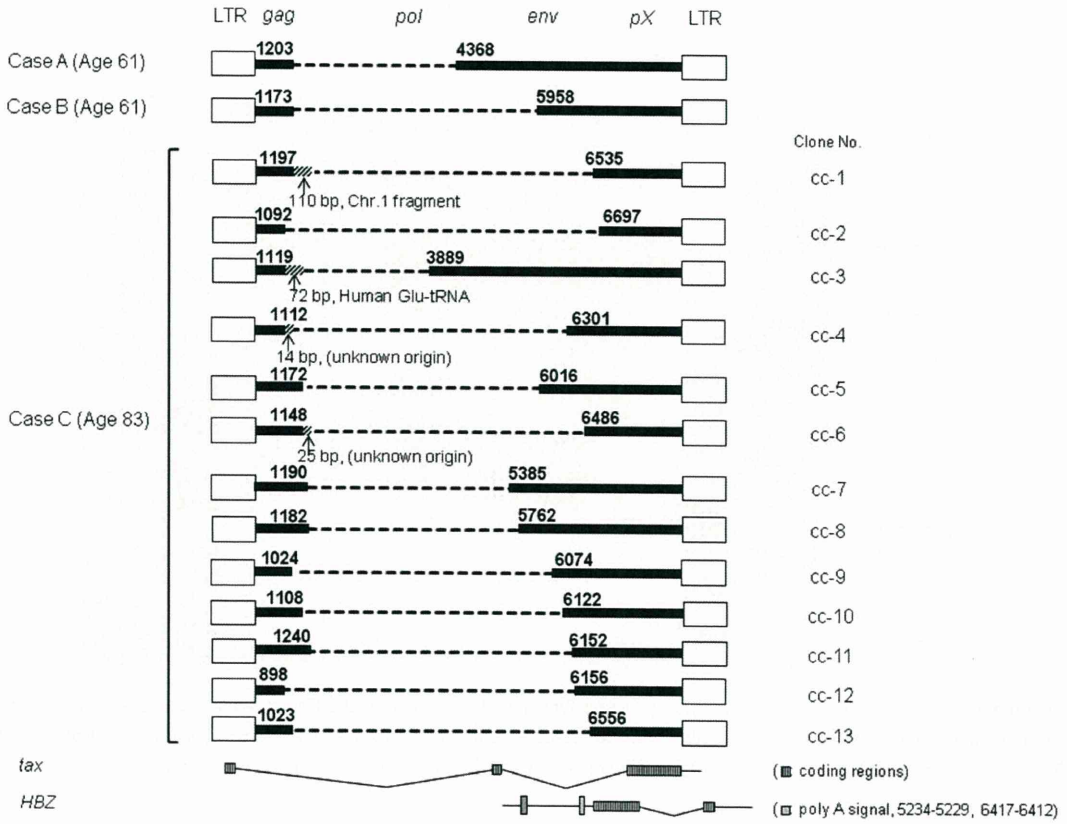

Figure 4. The schema of Type 1 defective provirus in Cases A, B and C. Dotted lines represent the defective regions of provirus. Splicing patterns of *tax* and *HBZ* genes are revealed. The nucleotide position numbers of human T-lymphotropic virus Type 1 (HTLV-1) provirus are same as those of Figure 1.

ages 61 and 70. The intensity of b-2 did not change during 9 years; however, that of b-1 increased at the age of 70. PVLs for the *pX* region increased (Fig. 2); however, the intensity of 2.9-kb Type 1 defective provirus by long PCR showed no change or decreased at age 70 (Fig. 3). Therefore, it was possible that clone b-2 represented the HTLV-1-infected cells with 2.9-kb Type 1 defective provirus detected by long PCR, and that another clone (b-1) of HTLV-1-infected cells with provirus, which was not detectable by long PCR, contributed to the increase of the PVLs in Case B. In Case C, one clone (c-1) was detected both at ages 73 and 84, and the other clone (c-2) was detected only at age 84. The intensity of c-1 was somewhat increased at the age of 84. The size of c-2 was ~7 kb and was considered to be a clone with defective provirus. However, clones c-1 and c-2 were not considered to be harboring Type 1 defective provirus because no band was observed to be increased in intensity at age 84 by long PCR (Fig. 3).

Discussion

To identify asymptomatic carriers, who have PBMCs harboring defective provirus with large deletions, PVLs of 208 asymptomatic HTLV-1 carriers were determined by real-time PCR using primers for the *pol* and *pX* regions. HTLV-1 *pX* region has been reported to be well conserved in the proviral genome.[14,16] Therefore, as expected, PVLs for the *pol* region were lower than those for the *pX* region. The carriers showing PVLs for the *pol* region, which were lower than those for the *pX* region, were considered to be candidates who have many PBMCs harboring defective provirus with large deletions of internal sequences. One hundred and eleven asymptomatic carriers showed relatively high PVLs (equal to or greater than 1.0 copy per 100 PBMCs). Seven showed low PVLs for the *pol* region (less than half of those for the *pX* region) among these 111 carriers. Four cases were excluded from further analysis because their low PVLs for the *pol* region were due to polymorphism of the proviral genome at the site of primer annealing. Three (Cases A, B and C) were considered as candidates for asymptomatic carriers, who have many HTLV-1-infected cells harboring defective provirus with large deletions. PVLs for the *pX* region increased in Cases A and B during follow-up for equal to or greater than 10 years. In contrast, PVLs for the *pol* region showed no change or decreased. These data suggested that the number

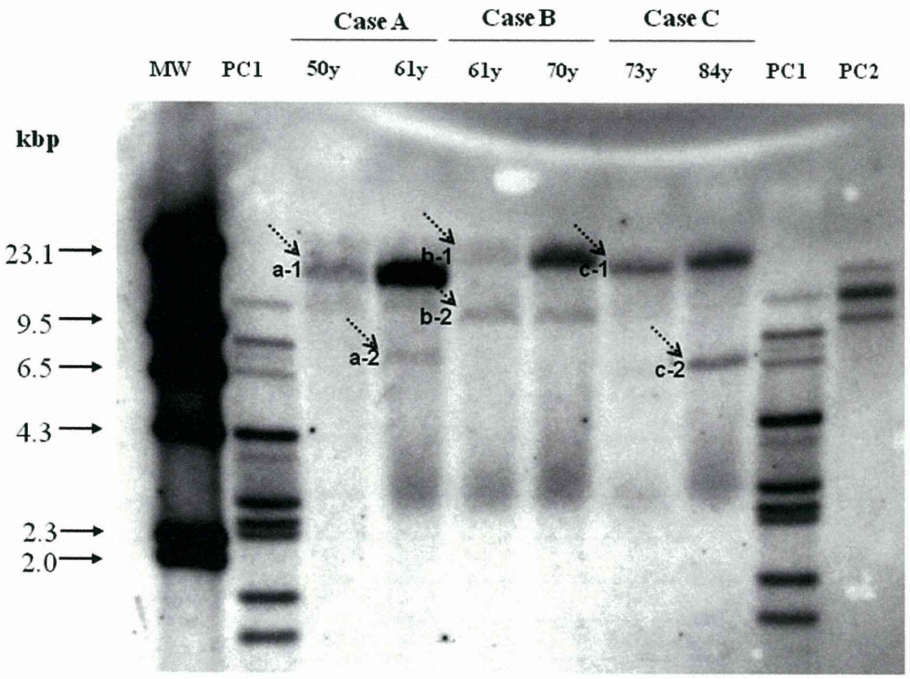

Figure 5. Southern blot analysis for human T-lymphotropic virus Type 1 (HTLV-1) provirus at different ages of Cases A, B and C. Arrows indicate predominant clones of HTLV-1-infected cells. PC1: DNA sample from HTLV-1-positive cell line, ST-1, which was digested with *Pst* I; PC2: DNA sample from HTLV-1-positive cell line, SO_4, which was digested with *Eco* RI; MW: molecular weight marker.

of HTLV-1-infected cells harboring defective provirus increased in Cases A and B.

Then, the defective provirus and the clonality of HTLV-1-infected cells were analyzed for the time-sequential samples from each subject. In Case A, a Type 1 defective provirus with a deletion of internal sequence of 3.2 kb was evident at the age of 50, and its intensity increased at age 61 (Fig. 3). Therefore, the increase of PVLs for the *pX* region, not for the *pol* region, was considered because of the clonal expansion of the HTLV-1-infected cells with this Type 1 defective provirus. Southern blot analysis showing the increased intensity of clone a-1 at age 61 supported this hypothesis. In Case B, a Type 1 defective provirus with the deletion of internal sequence of 4.8 kb was evident at age 61 (Fig. 3). The increase of PVLs for the *pX* region was not explained by the expansion of the HTLV-1-infected cells with this Type 1 defective provirus because the intensity of the PCR product for this defective provirus decreased at ages 72 and 73. Southern blot analysis showed two clones at the age of 61. Clone b-2 showed the same intensity at ages 61 and 70; however, clone b-1 increased in intensity at age 70. Therefore, the HTLV-1-infected cells harboring the Type 1 defective provirus in this case were more likely to belong to clone b-2. The increased PVLs at ages 70–73 were considered to be due to an increased number of HTLV-1-infected cells, which belonged to clone b-1. Clone b-1 was assumed to harbor the defective provirus because only a very weak band for complete provirus was detectable by long PCR in Case B. However, the defective provirus accounting for clone b-1 was not detectable by long PCR and could be a Type 2 defective provirus. Alternatively, a polymorphism of the DNA sequence in the site of primers of long PCR in 5′-LTR for clone b-1 may explain the absence of the band for defective provirus by long PCR. Therefore, it was considered that two major clones with defective provirus existed in Case B at age 61, and only clone b-1 survived as time passed. In Case C, several bands, which may have represented different Type 1 defective provirus, were detected by long PCR in addition to the band for the complete provirus at age 73. These defective proviruses were not consistently detectable at ages 73, 83 and 84. The intensity of the band for the HTLV-1-infected cells with complete provirus was also decreased as time passed. Therefore, maintenance of high PVLs at the age of 84 was not explained by these Type 1 defective proviruses alone. HTLV-1-infected cells with defective provirus, which was not detectable by long PCR in our study, might exist in Case C. In fact, Southern blot analysis showed increased intensity of clone c-1 and the appearance of new clone c-2 at age 84 (Fig. 5). The data from Cases A, B and C in our study suggested that HTLV-1-infected cells with certain types of defective provirus could be the predominant clones and persist for several years; however, the clones of HTLV-1-infected cells do not always survive for a long time in asymptomatic carriers.

Furukawa *et al.* reported that clonally proliferated cells infected with HTLV-1 were detected and were stable for from 4 months to 3 years in patients with HAM/TSP and their seropositive family members without showing any significant indication of ATL.[23] None of Cases A, B and C in our study showed any symptoms and data suggesting ATL- or HTLV-1-associated diseases, even at the end of the follow-up. Therefore, these carriers were judged not to have developed clinical ATL although they had high PVLs and clonal expansion of HTLV-1-infected cells with defective provirus. HTLV-1 Tax protein has been shown to promote the proliferation of infected cells.[13,24] On the other hand, Tax is also reported to be a good target for the host cellular immune response to HTLV-1.[25] HBZ protein was also reported to be important for the proliferation of HTLV-1-infected cells.[26–28] The proviral genome for *HBZ* gene, which is transcribed from 3′LTR, can be conserved even in the Type 2 defective provirus.[16,18] The Type 1 defective provirus found in Case A possessed internal deletion (positions 1203–4368). Theoretically, the expression of Tax and HBZ protein is not prevented by this internal deletion. Therefore, Tax and HBZ may have promoted the proliferation of HTLV-1-infected cells harboring this defective provirus although this proliferation might have been controlled by cytotoxic T-lymphocytes (CTL) through the recognition of Tax. At the same time, this defective provirus cannot express envelope and core proteins, which were also reported as the targets for CTL in HTLV-1 carriers.[29] Therefore, HTLV-1-infected cells harboring this defective provirus may be able to avoid attack from CTL more efficiently. In Case B, Type 1 defective provirus detected by long PCR possessed larger internal deletion (positions 1173–5958). Theoretically, the expression of Tax was prevented because of the deletion of the second exon of the *tax* gene in this defective provirus. It is not clear whether the loss of the expression of Tax protein was related to the decreased intensity of this clone at age 73. Theoretically, this Type 1 defective provirus was able to express HBZ protein because the provirus genome of *HBZ* gene was conserved. In Case C, 13 Type 1 defective proviruses were found at age 83. Twelve among these 13 clones (except clone cc-3 in Fig. 4) had large internal deletions, which theoretically prevent the expression of Tax. Moreover, 4 of 12 clones had the deletions, which theoretically prevent the expression of HBZ protein because of either the deficiencies of the coding regions of *HBZ* and/or deficiencies of two poly A signals (clone cc-1, −2, −6 and −13 in Fig. 4). These large deletions of defective provirus might account for clones not being consistently detectable during a long period in Case C.

In Cases B and C, the increase of PVLs in the time-sequential samples could not be explained by the existence of HTLV-1-infected cells with Type 1 defective provirus. The different clones of HTLV-1-infected cells with defective provirus, which was not detectable by the long-PCR used in our study, might exist in these cases. Clonal expansion of HTLV-1-infected cells with defective provirus, which does not express Tax protein and may not be recognized by the CTL, but which does promote the proliferation of HTLV-1-infected cells under the expression of HBZ protein, was possible. Indeed, HTLV-1-infected cells harboring Type 2 defective provirus were found more frequently in patients with ATL, suggesting a greater potential for leukemogenesis.[17,18]

In Case C, 4 of 13 Type 1 defective proviruses were found to have insertions of nonviral sequences (clone cc-1, −3, −4 and −6 in Fig. 4). Tamiya *et al.* also reported that insertion of a nonviral sequence (35 bp), which was derived from human proline transfer RNA, between the primer binding site and *env* region of HTLV-1 provirus in a patient with ATL.[16] They assumed that this nonviral sequence was inserted into the defective provirus during reverse transcription because human proline transfer RNA had the 16-bp homologous sequence with the 5′-region of HTLV-1. In our study, the DNA sequences of the inserted nonviral sequences in clone cc-1 and −3 were compared to the sequence of the 5′-region of HTLV-1. However, the homologous sequence was not found, and we could not clarify the mechanism of insertions of nonviral sequences in the defective provirus in Case C.

A major limitation of our study is that we were unable to identify genome sequences of Type 2 defective provirus, which possibly existed in Cases B and C, because of technical limitations. Further study to identify Type 2 defective provirus in asymptomatic carriers through improved methodology is necessary. In addition, the number of cases in which defective provirus was analyzed was small in our study. The analysis of more cases may clarify whether the HTLV-1-infected cells harboring the defective provirus have a growth advantage.

In our study, PVLs measured using primers for the *pol* region were less than those for the *pX* region in 208 asymptomatic HTLV-1 carriers. Analysis of seven carriers, who had relatively high PVLs for the *pX* region but much lower PVLs for the *pol* region, showed that they had HTLV-1-infected cells with polymorphism of proviral genome for the *pol* region or with defective provirus. All three asymptomatic HTLV-1 carriers, who had many HTLV-1-infected cells with defective provirus, showed high PVLs. The PVLs in two of the carriers increased markedly after a 10- to 12-year interval. This increase was considered to be due to the expansion of HTLV-1-infected cells with defective provirus. Accordingly, it is suggested that HTLV-1-infected cells with certain types of defective provirus can be predominant clones; however, not all predominant clones of HTLV-1-infected cells survive for a long time. Therefore, the detection of major clones of HTLV-1-infected cells may not always predict the development of ATL. Further study is necessary to clarify whether certain types of defective provirus are related to disease outcome such as ATL.

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Original Article

Suppressor of cytokine signal 3 and IL28 genetic variation predict the viral response to peginterferon and ribavirin

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Aim: The aim of this study was to investigate the relationship among the expression of suppressor of cytokine signaling 3 (SOCS 3) in the liver, the SNPs in the IL28B locus, and the outcome of interferon therapy.

Methods: Prior to interferon treatment, we immunostained 67 liver specimens from chronic hepatitis C (CHC) patients who were receiving peginterferon alpha-2b/ribavirin therapy for suppressor of cytokine signaling 3 (SOCS3), and compared the expression of SOCS3, IL28 polymorphisms and other clinical factors between the patients and compared their eventual outcomes.

Results: Significant differences between the low SOCS3 group and high SOCS3 group were found in age, as well as in the platelet, transaminase, gamma-glutamyl transpeptidase levels. The incidence of high SOCS3 was not significantly different between subjects with the TT genotype and the TG genotype (TT : TG = 71%:29%, $P = 0.250$). In a multivariate analysis, age (≥65 years old) (odds ratio 0.221 [0.120–0.966], $P = 0.045$), IL28B gene (genotype TT) (odds ratio 5.422 [1.254–23.617], $P = 0.024$) and SOCS3 (high) (odds ratio 0.308 [0.104–0.948], $P = 0.040$) were significant predictors of the interferon response. In patients with the TT genotype, those with low SOCS3 immunostaining showed a high sustained virological response (69%), while the sustained virological rate was low (27%) in the patients with high SOCS3 immunostaining.

Conclusions: Using a combination of the SOCS3 immunostained area in the liver and the expression of IL28B single nucleotide polymorphisms might be a useful predictor of hepatitis C virus clearance by interferon therapy.

Key words: hepatitis C virus, IL28B, interferon, suppressor of cytokine signaling 3

INTRODUCTION

APPROXIMATELY 200 MISSION people worldwide are infected with hepatitis C virus (HCV). In Japan, about 2 million people are chronically infected, and HCV is the leading cause of hepatocellular carcinoma (HCC). The current standard care for chronic hepatitis C (CHC) is a combination of peginterferon-α (PEG-IFN) and ribavirin. This treatment is effective in approximately 40–50% of CHC patients with a high viral load of genotype 1.[1–5] This therapy is costly and frequently associated with side effects. Therefore, predicting the outcome of interferon therapy is important.

Several factors, such as gender, body mass index, the presence of steatosis and liver fibrosis, drug adherence and viral factors including the serum quantity of HCV RNA and HCV genotype have been reported to be significantly associated with the treatment outcome.[2,6–11] Among viral factors, Akuta *et al.* recently reported that the substitution of the HCV core amino acid was a predictor for the effect of interferon and ribavirin combination therapy.[2,12] Among the host factors, recent reports showed that genetic variations near the IL28 gene (rs8099917, rs1297860) on chromosome 19 were predictors of the virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals

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with HCV, and also affected the clinical outcome, including spontaneous clearance of HCV.[13–15]

We previously reported that the expression of suppressor of cytokine signaling 3 (SOCS3), which is related to insulin resistance, impairs the response to interferon treatment and might be a useful predictor of HCV clearance by interferon therapy.[16]

In this study, we examined the relationship among the expression of SOCS 3 in the liver, single nucleotide polymorphisms (SNPs) in the IL28B locus, and the outcome of interferon therapy.

METHODS

NEEDLE BIOPSIES OF the liver were obtained from 67 patients with positive HCV antibodies prior to interferon treatment at Nagasaki University Hospital and National Hospital Organization (NHO) Nagasaki Medical Center. Twenty of 67 cases were also examined in a previous study.[16] All patients with genotype 1b received weekly injections of PEG-IFN. The clinical data of the patients are summarized in Table 1. Liver biopsy was performed by needle puncture for diagnostic purposes. The diagnosis of each case was independently confirmed histologically by liver pathologists according to the Japanese chronic hepatitis classification criteria (New Inuyama classification). According to these criteria, mild activity was defined as A0 or A1, severe activity as A2 or A3, mild fibrosis as F0 or F1, and severe fibrosis as F2, F3, or F4. Fatty changes in >5% of all areas were defined as steatosis.

Table 1 Clinical backgrounds of the patients

Age	56.8 ± 9.3
Gender	Male : Female = 37:30
BMI (kg/m^2)	23.5 ± 2.9
Viral load (KIU/mL)	2320 ± 1519
White blood cell (/uL)	5074 ± 1713
Hemoglobin (mg/dL)	14.1 ± 1.3
Platelet (×10^3/uL)	167.3 ± 75.6
AST (IU/L)	77.1 ± 45.2
ALT (IU/L)	101.2 ± 56.3
γGTP (IU/L)	70.6 ± 65.5
HCV core 70 wild	40 cases
HCV core 91 wild	50 cases
Steatosis (>5%)	37 cases
A (0–1:2–3)	36:31
F (0–1:2–4)	22:45

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, gamma-glutamyl transpeptidase; HCV, hepatitis C virus.

All patients received PEG-IFN (Schering-Plough, Tokyo, Japan) + ribavirin (Schering-Plough, Tokyo, Japan) therapy for 48 weeks. The patients who were treated with a dose of PEG-IFN or ribavirin reduced by more than 20% were excluded from the study. PEG-IFN (1.5 μg/kg) was administered once per week, and the ribavirin dose was titrated according to body weight. A sustained virological response (SVR) was defined as undetectable HCV RNA at 6 months after the end of interferon treatment.

Of 38 patients who could not achieve an end-of-treatment response, 28 patients required a re-elevation of their viral loads regardless of the fact that the HCV-RNA levels were temporarily negative, and 10 patients did not achieve an HCV negative result during the entire treatment period.

SOCS3 immunohistochemistry

All tissue samples were fixed in 10% neutral buffered formalin and then embedded in paraffin, and 4 μm thick serial sections were cut from each paraffin block. In the immunohistochemical study, an anti-SOCS3 antibody (dilution 1:100, Affinity BioRegents, Golden, CO, USA) was used for SOCS3. Immunohistochemistry was performed with the labeled streptavidin biotinylate antibody (LSAB) method and a commercially available kit (Histofine, SAB-PO(R); Nichirei Corporation, Tokyo, Japan). The area immunostained for SOCS 3 was divided according to the number of immunoreactive cells per unit area. Immunoreactive cases were classified as those with less than 30% of the hepatocellular cells stained (low SOCS3 group) and those with 30% or more of the cells stained (high SOCS3 group), because our previous study showed that staining of more than 30% of the area was a significant predictor of viral clearance.[16]

Genetic variation near the IL28B gene

Genotyping for replication was performed by use of the Invader assay or direct sequencing. In this study, genetic variation near the IL28B gene (rs8099917), which was previously reported to be a predictor of the virological response was investigated.[13]

Statistical analysis

The SPSS 9.0 for Windows statistical software program was used to assess correlations among multiple variables. When appropriate, clinical and laboratory data

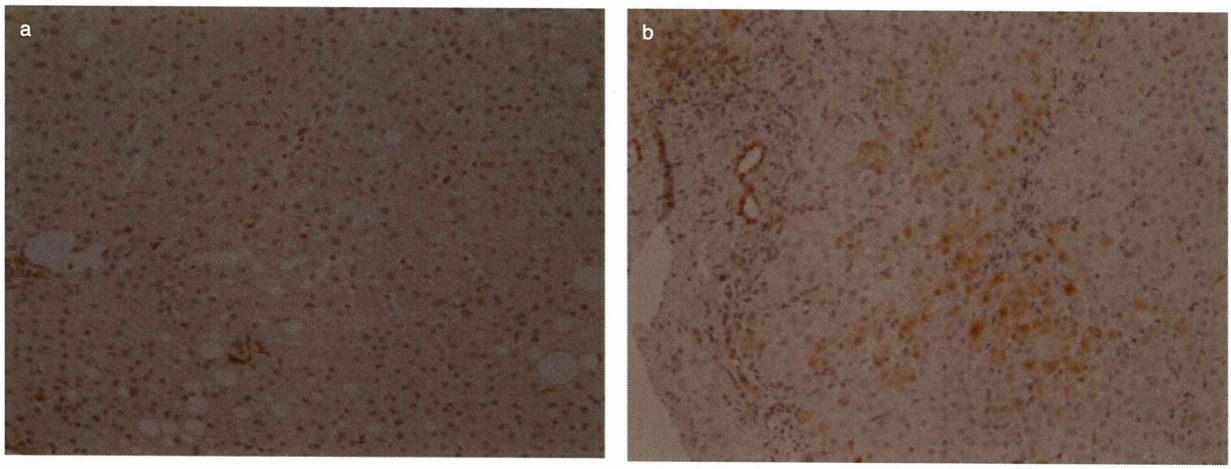

Figure 1 (a) This case showed less than 5% suppressor of cytokine signaling 3 (SOCS3) immunostained areas (low immunostaining). (b) This cases showed about 50% SOCS3 immunostaining areas (high immunostaining).

were compared with the Student's *t*-test or the Mann–Whitney test. A *P*-value of <0.05 was considered to be statistically significant.

RESULTS

Immunostaining of SOCS3 in the liver (Figs 1,2)

IMMUNOSTAINING FOR SOCS3 was mainly seen in the periportal area. Less than 30% SOCS3 immunostained areas were found in 36 cases (54%) and areas with 30% or more immunostaining for SOCS3 were found in 31 cases (46%).

The frequency and distribution of the SOCS3 expression are shown in (Fig. 2)

Correlation between SOCS3 immunostaining and clinicopathlogical factors

A significant difference between low and high SOCS3 groups was found in age (low : high = 54.5 ± 9.8:59.5 ± 8.1, $P = 0.028$), the levels of platelets (low : high = 189.5 ± 90.0:141.6 ± 41.3, $P = 0.009$), aspartate aminotransferase (AST) (low : high = 94.5. ± 56.0:62.1 ± 33.5, $P = 0.003$), alanine aminotransferase; (ALT) (low : high = 85.8 ± 52.4:119.0. ± 56.3, $P = 0.015$), gamma-glutamyl transpeptidase (γGTP) (low : high = 48.8 ± 53.5:94.7 ± 70.6, $P = 0.004$). The incidence of steatosis (low : high = 33%: 81%, $P = 0.001$), severe activity (low : high = 27%: 67%, $P = 0.001$) and sever fibrosis (low : high = 52%: 84%, $P = 0.006$) was significantly higher in the SOCS3 high

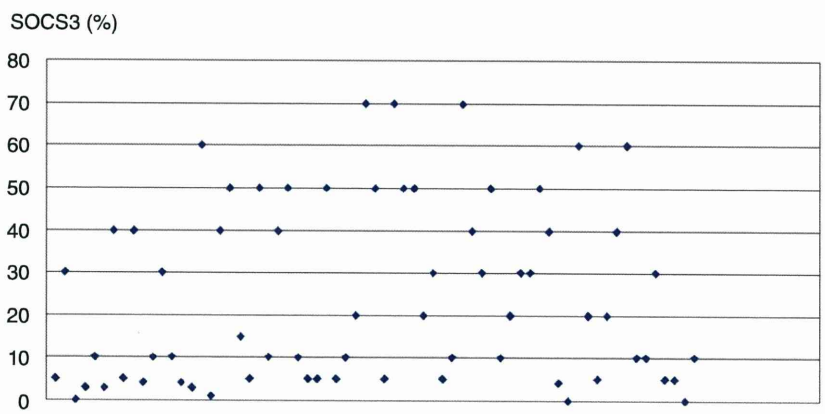

Figure 2 The distribution of the SOCS3 immunostaining area is shown.

Table 2 Comparison of the suppressor of cytokine signaling 3 (SOCS3) immunostaining groups

	SOCS3 high 31 cases	SOCS3 low 36cases	*P*-value
Age	59.5 ± 8.1	54.5 ± 9.8	0.028
Gender (male)	16 (53%)	21 (58%)	0.581
BMI (kg/m^2)	23.3 ± 2.2	23.6 ± 3.5	0.719
Viral load (KIU/mL)	2139 ± 1367	2475 ± 1950	0.427
White blood cell (/uL)	4935 ± 1386	5039 ± 1384	0.765
Hemoglobin (mg/dL)	14.1 ± 1.1	14.0 ± 1.3	0.570
Platelet (×10^3/uL)	141.6 ± 41.3	189.5 ± 90.0	0.009
AST (IU/L)	94.5. ± 56.0	62.1 ± 33.5	0.003
ALT (IU/L)	119.0 ± 56.3	85.8 ± 52.4	0.015
γGTP (IU/L)	94.7 ± 70.6	48.8 ± 53.5	0.004
Core 70 wild	17 (55%)	23 (63%)	0.451
Core 91 wild	23 (74%)	27 (75%)	0.939
Steatosis	25 (81%)	12 (33%)	0.001
Activity (severe)†	21 (67%)	10 (27%)	0.001
Fibrosis (severe)‡	26 (84%)	19 (52%)	0.006
IL28 TT rs8099917	22 (71%)	29 (80%)	0.358

†Severe activity was defined as A2 or A3.
‡Severe fibrosis was defined as F2, F3, or F4.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, gamma-glutamyl transpeptidase; HCV, hepatitis C virus.

immunostaining group than in the SOCS3 low immunostaining group. No significant difference was observed between the SOCS3 low and high groups in any of the other clinical factors (age, body mass index [BMI], viral load, white blood cell count, hemoglobin, substitution of the core 70, 91) (Table 2).

Comparison of SOCS3 expression and the genetic variation of IL28B gene

No significant difference in the genetic variation of the IL28 TT genotype was observed between the SOCS3 low and high immunostaining groups (low : high = 80%: 71%, $P = 0.250$).

Assessment of SOCS3 expression and genetic variation in IL28 as predictors of a sustained virological response

The age of patients in the non responder (NR) group was significantly higher than that in sustained virological response (SVR) group (SVR : NR = 52.3 ± 11.5: 59.6 ± 6.1, $P = 0.003$).

The incidence of the IL28 TT genotype was significantly lower, and that of SOCS3 high immunostaining group was significantly higher in the NR group than in the SVR group (Table 3).

As determined by a logistic regression analysis, the significant predictor of an SVR was high age (≥65 years old) (odds ratio 0.221 [0.120–0.966], $P = 0.045$), the IL28 TT genotype (odds ratio 5.422 [1.254–23.617], $P = 0.024$) and SOCS3 (high) (odds ratio 0.308 [0.104–0.948], $P = 0.040$) (Table 4).We found that two of nine (22%) patients with the IL28 TG genotype and SOCS3 high immunostaining showed a SVR, while one of seven (14%) patients with the IL28 TG genotype and SOCS3 low immunostaining, six of 22 (27%) patients with the IL28 TT genotype and SOCS3 high immunostaining, and 20 of 29 (69%) patients with the IL28 TG genotype and SOCS3 low immunostaining showed a SVR (Fig. 3).

DISCUSSION

RECENT IMPROVEMENTS IN the efficiency of antiviral therapy have led to approximately 50% of patients with HCV genotype 1 achieving sustained viral clearance.[1–5] However, some patients are refractory to interferon therapy. A recent study reported that the presence of genetic variation near the IL28B gene (rs8099917, rs1297860) can be used as a pretreatment predictor of virlogical response to a 48-week PEG-IFN plus combination therapy in patients with HCV geno-

Table 3 Factors associated with the response to peginterferon-α (PEG-IFN) and ribavirin

	SVR 29 cases	NR 38 cases	*P*-value
Age	52.8 ± 11.0	59.8 ± 6.4	0.002
Gender (male)	17 (58%)	20 (52%)	0.625
BMI (kg/m^2)	23.9 ± 3.1	22.9 ± 3.1	0.190
Viral load (KIU/mL)	2188 ± 1764	2420 ± 1689	0.587
White blood cell (/uL)	4816 ± 1427	5225 ± 1287	0.242
Hemoglobin (mg/dL)	14.1 ± 1.1	14.0 ± 1.3	0.626
Platelet (×10^3/uL)	176.5 ± 52.8	160.3 ± 89.2	0.350
AST (IU/L)	75.5 ± 36.1	78.3 ± 51.5	0.795
ALT (IU/L)	108.9 ± 56.8	95.3 ± 56.0	0.333
γGTP (IU/L)	63.9 ± 61.9	75.7 ± 68.6	0.464
Core 70 wild	20 (69%)	20 (53%)	0.176
Core 91 wild	21 (72%)	29 (71%)	0.173
IL28 TT rs8099917	26 (90%)	25 (65%)	0.022
steatosis	14 (47%)	23 (61%)	0.452
Activity (severe)†	10 (34%)	21 (64%)	0.091
Fibrosis (severe)‡	18 (62%)	27 (71%)	0.437
SOCS3 (Positive)	8 (27%)	23 (61%)	0.015

†Severe activity was defined as A2 or A3.
‡Severe fibrosis was defined as F2, F3, or F4.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, gamma-glutamyl transpeptidase; HCV, hepatitis C virus; NR, non responder; SOCS3, suppressor of cytokine signal 3; SVR, sustained virological response.

type 1.[13–15] We previously reported that SOCS3 was a factor associated with the response to PEG-IFN treatment.[16] We compared these factors and clarified their usefulness as predictors of PEG-IFN plus combination therapy.

In the laboratory data from our patients, a significant difference between the groups with weak and strong SOCS3 staining was found in the level of AST, ALT, and platelets. These laboratory data suggested that the SOCS3 immunostained area was significantly associated with the presence of inflammation and the fibrosis stage. Indeed, in a pathological study, the inflammation and fibrosis stage were significantly different between the low and high SOCS3 immunostaining groups. This finding was consistent with our previous study that showed that the SOCS3 immunostained area was influenced by inflammation and the fibrosis stage.[16]

Table 4 Results of a multilogistic regression analysis

	Odds ratio	*P*-value
Age (>65 years)	0.221 (0.120–0.966)	0.045
IL28 TT	5.422 (1.254–23.617)	0.024
SOCS3 (low)	0.308 (0.104–0.948)	0.040

SOCS3, suppressor of cytokine signal 3.

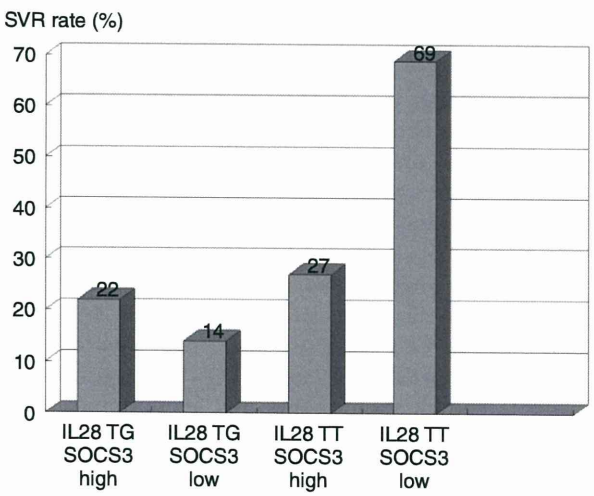

Figure 3 A total of 12.5% of patients with IL28 TG and suppressor of cytokine signaling 3 (SOCS3) high immunostaining showed a sustained virological response (SVR), 20% of patients with IL28 TG and SOCS3 low immunostaining, 31% of patients with IL28 TT and SOCS3 high immunostaining, and 68% of patients with IL28 TG and SOCS3 low immunostaining showed a SVR.

Moreover, a significant difference between the low and high SOCS3 groups was also found in the level of γGTP. Several previous reports showed that the level of γGTP was correlated with steatosis in the liver.[7,17] In this study, the presence of steatosis also was significantly different in the low and high SOCS3 immunostaining groups. Together with our results, these results demonstrated that the SOCS3 immunostained area in the liver was associated with obesity, insulin resistance, and hepatic steatosis.[18,19]

Although recent reports showed that genetic variation of IL28B was also associated with liver inflammation and fibrosis,[20] this was not associated with the SOCS3 immunostained area in the present study. The SOCS3 proteins are known for their role as negative regulators and inhibitors of Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling, where they mediate a classical negative feedback loop in the IFN-α/β receptor signaling pathway.[21,22] The mechanism that leads to the association between genetic variation of IL28B and the effect of interferon therapy is clear, because it has been demonstrated that IL28B inhibits hepatitis C virus replication through the JAK-STAT pathway.[23] Taken together, both the SOCS3 immunostained area and IL28B polymorphisms were associated with the JAK-STAT pathway, but the different factors might interfere with JAK-STAT signaling in different ways.

The NR rate to combination PEG-IFN plus ribavirin therapy in patients with the non-TT genotype was 10–20%. The value of NR for the prediction of the genetic variation of IL28B was therefore very high. On the other hand, the SVR rate in patients with the TG genotype was about 50%. The value of SVR prediction based only on the genetic variation of IL28B was therefore not as strong for this genotype.

The substitution of core amino acids was also reported to be a predictive factor for the response to interferon therapy and was significantly associated with the genetic variation of IL28B.[24] On the other hand, the SOCS3 immunostained area was independent of both of these factors. Thus, we suggested that using a combination of the SOCS3 immunostained area with the IL28B genotype can provide the best prediction of the response to PEG-IFN plus ribavirin therapy.

Indeed, in TT genotype patients, the SVR rate in the SOCS3 weak group was about 70%, and NVR rate in the SOCS3 low immunostained group was 27%. If a liver biopsy was performed, immunostaining for SOCS3 was easy, and provided a useful predictor of the response to interferon therapy.

Our study has some limitations. Our sample size was too small. Further large-scale studies are necessary to confirm the present results and to provide a better understanding of the interactions between the SOCS3 immunostained area and the genetic variation of IL28B.

In conclusion, a combination of the SOCS3 immunostained area in the liver and the assessment of the genetic variation of IL28B seem to be good predictors of the response to PEG-IFN plus ribavirin therapy.

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Living Donor Liver Transplantations in HIV- and Hepatitis C Virus-Coinfected Hemophiliacs: Experience in a Single Center

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Background. Although almost all human immunodeficiency virus (HIV)-infected Japanese hemophiliacs are coinfected with hepatitis C virus (HCV), the outcome of living donor liver transplantation (LDLT) in such patients in terms of survival rate, perioperative complications, and recovery of coagulation activity is poorly understood.
Patients and Methods. Six HIV-positive hemophiliacs underwent LDLT for HCV-associated advanced cirrhosis. The mean CD4 T-cell count at transplantation was 376±227/μL.
Results. The 1-, 3-, and 5-year survival rates were 66%, 66%, and 50%, respectively. Fatal perioperative bleeding related to hemophilia was not observed. Two patients died within 6 months after transplantation due to graft failure. HIV infection was well controlled in all patients who survived longer than 6 months. Two patients (genotype 2a and 2+3a) achieved a sustained viral response and both of them were alive at the end of follow-up period, whereas one patient (genotype 1a+1b) died of decompensated cirrhosis 4 years after transplantation due to recurrent HCV infection.
Conclusions. HIV/HCV-coinfected hemophiliacs can safely undergo LDLT. Hemophilia was clinically cured after successful transplantation. A good outcome can be expected as long as postoperative hepatitis C is controlled with interferon/ribavirin combination therapy.

Keywords: Hepatitis C virus, Living donor liver transplantation, HIV, HAART.

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Because of the availability of highly active antiretroviral therapy (HAART), the life expectancy of patients infected with human immunodeficiency virus (HIV) has dramatically improved (*1*). Death from opportunistic infections has decreased and, as the result, non-acquired immune deficiency syndrome (AIDS)-defining complications such as hepatic diseases, cardiovascular diseases, or non-AIDS malignancies have emerged as the most important problems (*2, 3*).

Hepatitis C virus (HCV) and HIV often coinfect due to their shared route of transmission. A recent report indicated that approximately 20% of HIV-infected people in Japan are coinfected with HCV (*4*), a large proportion of whom are hemophiliacs. Approximately 1500 hemophiliacs were infected with HIV through non heat-treated concentrated coagulation factor administration between 1981 and 1985, and 98% of them were also infected with HCV. The coexistence of HIV infection with HCV accelerates the progression of liver fibrosis (*5*) and attenuates the efficacy of interferon (IFN) treatment for HCV (*6, 7*). A considerable number of such coinfected patients suffer from decompensated cirrhosis or hepatocellular carcinoma (HCC) (*8*). In the HAART era, AIDS-related death is gradually decreasing (*9*) and HCV-

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related liver diseases have become the leading cause of death in Japanese hemophiliacs (*10*).

The only curative treatment for end-stage liver disease is liver transplantation. In the pre-HAART era, HIV infection was considered an absolute or relative contraindication for transplantation. Several cases were reported during that period (*11*, *12*), but the outcomes were not always satisfactory. In the HAART era, more than 50 cases of HIV-positive liver transplantation have been reported (*13–21*), and survival after liver transplantation seems to be more promising.

The absolute number of deceased donor livers in Japan is small, and living donor liver transplantation (LDLT) is the mainstay of liver transplantation. We reported the first LDLT in an HIV-positive hemophiliac in 2002 (*22*). Here, we present a series of six cases of LDLT in HIV/HCV-coinfected hemophiliacs performed at the University of Tokyo Hospital between 2001 and 2004.

RESULTS

Survival

The 1-, 3-, and 5-year survival rates were 66%, 66%, and 50%, respectively. Two patients (cases 2 and 5) died on postoperative day (POD) 99 and 156, respectively. The causes of early death were graft failure and bleeding from cytomegalovirus (CMV) enteritis (case 2) and graft failure suspected to be cholestatic hepatitis (case 5). One patient died 50 months after LDLT due to recurrent HCV-related cirrhosis.

Results of Antiviral Therapy for Recurrent Hepatitis C in the Graft

After LDLT, all but one (case 2) patients received combination therapy with IFN (standard or pegylated form) and ribavirin. Case 3 was treated for biopsy-proven recurrent hepatitis C, whereas the other four were treated preemptively (started on POD, 10–70 days). Duration of anti-HCV therapy was 12 months in case early viral response was achieved. Cases 1 and 3 achieved sustained viral response (SVR). Case 3 suffered from HCV-related cholestatic hepatitis on POD 38, which responded well to combination therapy with IFN and ribavirin and he eventually achieved SVR. The other patients did not achieve SVR. Cases 4 and 6 showed a biochemical response and were on maintenance antiviral therapy. In case 6, tacrolimus was switched to cyclosporine A 15 months after LDLT to suppress HCV replication. This lead to a transient 10-fold decrease in HCV-RNA, but it returned to the previous value within several months.

Results of Antiretroviral Therapy After LDLT

Antiretroviral therapy was transiently terminated during the perioperative period. The timing of reintroduction was individualized according to the CD4 count, HIV viral load, general status such as surgical complication and the result of liver function tests. One patient (case 1) has continued to maintain a high CD4 count without antiretroviral therapy. One patient (case 2) died before antiretroviral reintroduction.

The remaining four patients started antiretroviral therapy at a median of 56.5 days after LDLT (range, 43–485 days). The choice of the antiretroviral drug was individualized according to each patient's antiretroviral history and accumulated resistance mutations. A protease inhibitor-based combination was selected in all cases. All but one patient (case 5) tolerated antiretroviral therapy and had an excellent response. The blood concentration of the immunosuppressant increased drastically from the first day of protease inhibitor administration, which was controlled by close monitoring and dosage modification.

Elevation of serum alkaline-phosphatase and gamma-glutanyl-transpeptidase values was observed in all patients after antiretroviral reintroduction. Other significant adverse effects include severe allergic reaction to lamivudine (case 3) and liver failure, which was clinically diagnosed to be cholestatic hepatitis as an immune reconstitution inflammatory syndrome against HCV (case 5).

One patient (case 3) developed Burkitt leukemia 38 months after LDLT. His CD4 count at that time was 480/μL and HIV-RNA was undetectable. Combination chemotherapy using cyclophosphamide, vincristine, doxorubicin, and dexamethasone (*23*) was effective, and he eventually achieved complete remission. Other opportunistic infections included multiple abscess formation at the surgical site in two patients (case 2 by methicillin-resistant *Staphylococcus aureus* and case 5 by multi-drug resistant *Pseudomonas aeruginosa*). Positive CMV antigenemia was observed in all cases. However, only one patient (case 2) presented with clinically overt organ damage.

Restoration of Coagulation After LDLT

Except for case 5, replacement became unnecessary within 1 week after operation. In case 5, in addition to insufficient endogenous coagulation factor production, re-operation was necessary several times, and the coagulation factor replacement could not be withdrawn. Cases 2 and 6 again required coagulation factor replacement after graft failure became apparent.

Outcome of the Donors

All donors were alive without major complications at the point of analysis. Two donors were considered obligate carriers of hemophilia and one of them (donor of case 5) showed relatively low coagulation activity, but none of the donors experienced abnormal bleeding requiring coagulation factor administration. The donor of case 5 experienced transient decrease in factor IX activity after liver resection. However, the value of coagulation activity recovered without supplementation.

DISCUSSION

Recurrence of hepatitis C is the most important problem in treating HCV-positive hemophiliac patients. Recent reports indicate that HIV/HCV-coinfected liver recipients have a relatively lower survival rate than HCV-monoinfected liver recipients, although the difference is not significant. In our series, two of three deaths were related to recurrent HCV, and two patients experienced fibrosing cholestatic hepatitis. Cholestatic hepatitis is characterized by a high rate of HCV replication and a paucity of inflammatory activity, and the risk might increase in LDLT recipients (*24*, *25*). In our center, IFN therapy is usually introduced preemptively as soon as possible. In our series, two cases infected with non-1b virus achieved SVR, whereas others did not achieve SVR. A report demonstrated the effectiveness of maintenance therapy with

pegylated (PEG)-IFN plus ribavirin (*26*), but this efficacy was not apparent in our series. Combination antiviral therapy with protease and polymerase inhibitors may improve the treatment results in the future.

With regard to HIV infection, when to restart antiretroviral therapy after LDLT has remained a question. Hemophiliacs often have a long-term treatment history. Five of six cases had a multiple history of treatment failure, and as a result, only one or two reliable antiretroviral combinations were available to each patient in that era. Protease inhibitors, key drugs for successful HIV suppression in such cases have a potential risk of liver toxicity, especially in those with HCV coinfection (*27*). Unlike whole liver transplantation, the initial graft size is relatively small in LDLT. The graft gradually increases its volume within several weeks after transplantation, and an unfavorable effect of antiretroviral treatment on graft growth during this period is a concern. Moreover, unintended treatment interruption due to early phase complications may result in further accumulation of resistance-associated mutations. Taking these issues into account, we delayed starting antiretroviral therapy until at least 4 weeks after LDLT. It is obvious, however, that earlier antiretroviral reintroduction has more benefit toward reducing opportunistic infections and improving the result of anti-HCV therapy after LDLT. The effectiveness and safety of a new class antiretrovirals, raltegravir (*28*), and enfuvirtide (*29*), were recently reported, and these compounds may play an important role in the management of HIV-infected split-graft recipients.

In our series, the immunosuppressant trough level was targeted to the same level as that in HIV-negative cases. It is not known, however, whether HIV-infected patients, particularly those with a relatively lower CD4 cell count, need the same blood level of immunosuppressants. Moreover, the CD4 cell count, may not act as accurate surrogate marker for immune function in those taking an immunosuppressant or steroid. In case 2, recurrent bleeding from CMV intestinal ulcer eventually led to death after immunosuppression was intensified to treat severe graft rejection. In this case, antiretroviral therapy could not be reintroduced because of severe liver damage, which might enhance excess immunosuppression. A more precise indicator than CD4 count and immunosuppressant level is needed. Dose modification of immunosuppressive drugs using an immune function assay (*30*) may contribute to more precise management, especially in HIV-coinfected patients.

A considerable number of HIV/HCV-coinfected patients are suffering from decompensated cirrhosis or HCC (*8*), and some of them are potential candidates for future liver transplantation. The shortage of deceased donor liver grafts is a major problem worldwide. LDLT can overcome such a problem. Clearly, regenerative medicine will have an important role in this field in the future. Those patients who are already in a cirrhotic state, however, cannot wait for such an innovative modality to be established. In our series, all patients who tolerated antiretroviral therapy achieved good HIV control, and those who cleared HCV survived long. Clinical cure of hemophilia after successful transplantation drastically improved the patients' quality of life. Cure of hemophilia also lead to considerable cost reduction. LDLT continues to have an important role in HIV-infected hemophiliacs.

MATERIALS AND METHODS

From April 2001 to October 2004, nine HIV/HCV-coinfected patients were referred to the University of Tokyo hospital for LDLT. The indication was HCV-related end-stage liver disease.

HIV-positive patients should meet the same standard criteria for liver transplantation as HIV-negative patients. The criteria for accepting candidates for LDLT were absolute CD4 T lymphocyte count more than 200/μL, or more than 14% CD4 proportion to total lymphocytes when hypersplenism-related leukocytopenia was considered the cause of an apparent decrease in the CD4 count. Undetectable HIV RNA was not required as long as effective HIV suppression was expected after transplantation. Exclusion criteria related to HIV infection were active AIDS-defining diseases except for esophageal candidiasis. All cases were approved by the ethics committee at the University of Tokyo. Donor was selected from those with spontaneous will and within the third-degree consanguinity of the patient. Those with abnormal coagulation values were excluded from candidate for the donor.

Two patients did not meet the criteria (one with concomitant uncontrollable fungal infection and one without appropriate donor). One patient retracted consent before operation. Finally, six HIV/HCV-coinfected hemophiliacs underwent LDLT. Two patients were transplanted emergently (within 2 weeks after referral) because of progressive hepatic encephalopathy and hepatorenal syndrome. None of the patients had concomitant active hepatitis B, HCC, or other malignancies. The patient characteristics are summarized in Table 1.

The appropriate type of concentrated coagulation factor was administered during the perioperative period. Concentrated coagulation factor was administered as a bolus just before the operation to achieve 100% coagulating

TABLE 1. Patient characteristics at LDLT and outcome

Case	Age/ sex	Type of hemophilia	HCV genotype	HCV-RNA at LDLT (KIU/mL)	HIV load (copy/mL)	MELD at LDLT	CCr	HTN/ DM	BMI	Graft	Graft size (%SLV)	ACR	CMV	Survival (mo)	Donor
1	41M	B	2a	3	UD	23	24	N/N	19.1	Right	66	0	1	Alive (115)	Brother
2	28M	A	2a, 2b	1410	6.2×10^4	15	76	N/N	23.4	Right	57	2	2	Died (3)	Mother
3	30M	A	1b, 3a	740	3.2×10^4	15	78	N/N	21.5	Right	42	1	2	Alive (96)	Mother
4	38M	A	1b, 3a	200	UD	34	69	N/N	20.0	Right	47	1	1	Alive (82)	Sister
5	31M	B	1a	747	2.6×10^4	18	72	N/N	24.3	Right	47	2	3	Died (5)	Mother
6	32M	B	1a, 1b	41	UD	48	62	N/N	25.2	Right	63	0	0	Died (50)	Father

HCV, hepatitis C virus; LDLT, living donor liver transplantation; HIV, human immunodeficiency virus; MELD, model for end-stage liver disease; CCr, creatine clearance; HTN, hypertension; DM, diabetes mellitus; BMI, body mass index; SLV, standard liver volume; ACR, acute cellular rejection; CMV, cytomegalovirus; UD, undetectable.

factor activity, followed by continuous infusion to maintain greater than 80% activity during the operation. Fresh-frozen plasma was also replaced. Initial dosage of the coagulation factor was calculated based on the results of preoperative pharmacokinetic studies, and the rate of continuous infusion was adjusted as necessary by periodical monitoring of coagulation factor activity.

Tacrolimus and steroids based immunosuppression was planned as previously described (*31*). The target tacrolimus trough level was same as that for the HIV-negative population. Moderate to severe rejection was treated with pulse steroids±mycophenolate mofetil.

The preoperative HCV-RNA value was positive in all subjects. The HCV genotype is listed in Table 1. All patients underwent concomitant splenectomy (*32*). Preemptive anti-HCV therapy with IFN (standard or pegylated form) plus ribavirin was planned after LDLT (*33*). Postoperative CMV reactivation was monitored using a pp65 antigen detecting method (CMV antigenemia), and a positive result was preemptively treated with ganciclovir (*34*) or valganciclovir.

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EDITORIAL

Liver transplantation in HCV/HIV positive patients

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Abstract

Since the introduction of highly active antiretroviral therapy (HAART) in 1996 for human immunodeficiency virus (HIV)-infected patients, the incidence of liver diseases secondary to co-infection with hepatitis C has increased. Although data on the outcome of liver transplantation in HIV-infected recipients is limited, the overall results to date seem to be comparable to that in non-HIV-infected recipients. Liver transplant centers are now accepting HIV-infected individuals as organ recipients. Post-transplantation HIV replication is controlled by HAART. Hepatitis C re-infection of the liver graft, however, remains an important problem because cirrhotic changes of the liver graft may be more rapid in HIV-infected recipients. Interactions between the HAART components and immunosuppressive drugs influence drug metabolism and therefore meticulous monitoring of drug blood level concentrations is required. The risk of opportunistic infection in HIV-positive transplant patients seems to be similar to that in HIV-negative transplant recipients.

Key words: Hepatitis C virus; Human immunodeficiency virus; Living donor liver transplantation; Interferon; Rivabirin

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INTRODUCTION

Liver transplantation is an established standard therapeutic option for end-stage liver disease (ESLD) with a 1 year survival rate exceeding 80%[1]. Before the introduction of highly active antiretroviral therapy (HAART) in 1996, the results of transplantation for patients with human immunodeficiency virus (HIV) were poor[2-5] and HIV was considered a contraindication in most centers[6-8]. The 3 year survival rates were around 45% and the most frequent cause of death was acquired immune deficiency syndrome (AIDS)[2-5].

Since the introduction of HAART, HIV-related morbidity and mortality have dramatically decreased from 20-30 to 1.5-2.5 per 100 person-years[9,10]. In the setting of the improved prognosis in the HIV-infected population, the focus has turned to the morbidity and mortality associated with hepatitis C (HCV) and ESLD[11-15]. As HAART has changed HIV infection to a chronic condition, transplant centers around the world are slowly becoming less reluctant to accept HIV-positive patients as transplant recipients for both medical and ethical reasons[16-19] and liver transplantation is now considered a possible therapeutic option for these patients. The aim of the present review is to explore the new indications for liver transplantation in HIV/HCV co-infected patients.

EPIDEMIOLOGY OF HIV AND HAART

An estimated 33 million persons are currently living with HIV infection worldwide, including 16 million women and 2 million children less than 15 years of age[20]. In 2008, 2 million adults and 0.4 million children were newly infected and 2 million died from HIV infection. HIV infection is transmitted *via* heterosexual contact, intravenous drug use and homosexual contact.

Liver-related diseases among HIV-infected persons are caused by hepatitis B virus and/or HCV co-infection, hepatotoxic medication, alcohol and illegal drug use. Of these, co-infection with HCV is the most frequent cause of liver disease in HIV patients and substantially contributes to morbidity and mortality[21,22]. HIV and HCV usually share common routes of transmission. Approximately 4 to 5 million HIV-infected persons are co-infected with HCV. The prevalence of co-infection differs by geographical region and by patient demographical and behavioral characteristics ranging from 1%-5% in persons who acquired HIV infection by heterosexual or homosexual contact to 70%-95% in patients with current or former injecting drug use and transfused hemophiliac patients[23].

HCV MANAGEMENT BEFORE TRANSPLANTATION

There are several challenges in the management of HIV/HCV co-infected patients who require transplantation. The first is to minimize mortality while on the organ transplant waiting list because such patients frequently have a more rapid progression from the first decompensation to death. The interval between the first manifestation of liver decompensation and death is 16 mo for HIV/HVC co-infected versus 48 mo for HIV mono-infected patients. Prognostic factors after first decompensation include age, severity of liver disease [model for end-stage liver disease (MELD) score] and the nature of the decompensation event[24].

HCV/HIV co-infections adversely affect each other: HIV infection accelerates the progression of HCV disease by increasing HCV viremia, causing cellular immunodeficiency, increasing the risk of liver fibrosis and cirrhosis and leading to the rare fibrosing cholestatic hepatitis[25,26]. HCV may adversely affect the course of HIV infection[27,28] by reducing the efficacy of antiretroviral treatment and increasing the rate of antiretroviral medication-related hepatotoxicity[25,29]. The effect of antiretroviral therapy to reduce liver-related mortality in HCV co-infected persons is controversial[10,30]. Some co-infected persons are not eligible for HCV treatment due to somatic or psychosocial comorbidities, contraindications for HCV drugs and decompensated cirrhosis[31].

INDICATIONS FOR TRANSPLANTATION

Table 1 showed the inclusion criteria for liver transplantation. The acceptance criteria for liver transplantation in HIV-positive recipients continue to evolve with increased experience with the co-infected population. In deceased donor liver transplantation, there are ethical concerns regarding the use of a scarce resource for a group of recipients with unknown survival duration. The acceptance criteria have gradually expanded, however, based on acceptable initial results[32-34].

The best timing for liver transplantation from the point of view of liver damage (Child-Pugh or MELD) is unknown. Pre-transplant survival for liver candidates is reported to be poorer in HIV-infected individuals compared with others awaiting liver transplantation, despite equivalent MELD scores[24,35]. In one study, significantly fewer HIV patients (26% of 58) survived on a liver transplant waiting list compared to candidates without HIV (63% of 860)[36]. Although more rapid deterioration in HIV co-infected patients may be the cause[37], death in that study was not associated with MELD, viral load, CD4, ability to tolerate medications or HCV progression. Further studies are necessary to understand the risk factors for death in HIV-positive patients on the liver transplant waiting list[38].

The inclusion criteria of an NIH sponsored study in the USA[39] are AIDS-related opportunistic infections or cancers that are resolved by sufficient treatment prior to transplant. CD4 counts should be greater than 100/mL for those without a history of opportunistic infection and greater than 200/mL for those with a history of opportunistic infection completely treated before transplantation. These requirements may be applied 3 to 4 mo prior to transplantation. Opportunistic infections include chronic cryptosporidiosis, progressive multifocal leukoencephalopathy and multi-drug resistant systemic fungal infections. Patients with a history of AIDS-associated lymphoma should be excluded. Most clinical trials include individuals with a history of resolved cutaneous Kaposi's sarcoma if a recent high-resolution computed tomography scan reveals no evidence of pulmonary lesions[40].

In a Spanish consensus statement[41], the criteria are similar except for the requirement of CD4 cell counts greater than 350/mL in patients that do not fulfill the HAART criteria. Ragni *et al*[32] noted that cumulative survival among HIV-positive recipients is similar to that of age- and race-comparable HIV-negative recipients.

Another potential conflict regarding the absolute requirement for a CD4 cell count greater than 100/mL relates to the use of interferon therapy[42]. In a recent series at Paul Brousse Hospital[42], CD4 cell counts greater than 150/mL were observed in 7 patients and 6 of these 7 patients developed severe chronic HCV. The use of interferon can cause a transient decrease in CD4 cell counts. The absolute CD4 cell count prior to interferon therapy should be taken into account in the decision regarding liver transplantation.

HIV-RNA should be undetectable at transplantation. Unfortunately, however, most recipients cannot tolerate HAART therapy due to its hepatotoxicity. When an undetectable HIV viral load is not achievable, an experienced HIV clinician should predict the ability to