RT step (to detect contaminating DNA) and reactions without primers (to check for self-priming). For the detection of HBV DNA, total DNA was extracted from liver tissue using a commercially available kit (SMI test EX R and D, Sumitomo Metal Industries, Tokyo, Japan). Purified total hepatic DNA was resuspended in 500 µL of distilled water. A 25-µL aliquot of DNA solution was subjected to real-time detection PCR using set 2 primers and set 3 primers. To avoid contamination in all PCR assays, the contamination avoidance measures of Kwok and Higuchi [1989] were strictly applied throughout, and positive and negative controls were used.

### Histological Evaluation

Liver biopsy specimens were fixed in formalin and embedded in paraffin for routine staining with hematoxylin-eosin. All specimens were examined by the same experienced pathologist (K.K.), who was unaware of the clinical, serological and virological data. Biopsy specimens were evaluated semiquantitatively by the histological activity index (HAI) described by Knodell et al. [1981].

## RESULTS

Figure 1 shows the clinical, virological, and histological course of the three chronic liver disease patients (patients 1, 2, and 3) with spontaneous circulating HCV clearance. They had undergone laparoscopy for the diagnosis of chronic non-A, non-B liver disease 6–11 years before their HCV-seropositive states without viremia were found in 1990–1991. After the discovery of HCV, they were positive persistently for HCV antibody by RIBA-3 over 8–9 years but remained negative for serum HCV RNA. Patient 1 showed slight serum ALT elevation (58 U/L, normal value ≤33 U/L) at laparoscopy in 1984. Histological findings demonstrated that she had moderate chronic hepatitis with bridging fibrosis (Fig. 2A). The HAI scores of categories I (piecemeal necrosis), II (lobular necrosis and inflammation), III (portal inflammation), and IV (fibrosis) were 3, 3, 4, and 3, respectively, and portal lymphoid aggregates characteristic for HCV infection were observed. During a 15-year follow-up period, the slight elevation of serum ALT activity was normalized. The levels of serum albumin, cholinesterase activity, and platelet count remained normal over the entire follow-up period. At the second liver biopsy in 1995, the histological findings were improved markedly (Fig. 2B). Although minimal chronic hepatitis remained, no fibrosis was seen. The HAI categories I, II, III, and IV were scored 0, 1, 1, and 0, respectively.

Laparoscopic findings in 1982 showed that patient 2 had established cirrhosis with an obviously nodular liver surface. Although his liver biopsy specimen was not available, he also had symptoms of liver cirrhosis including esophageal varices. Over a 17-year follow-up period, serum ALT levels remained normal or near-normal. The levels of serum albumin and cholinesterase activity were also within the normal range although a slight decrease of platelet count persisted during follow-up. Liver histology both in 1998 and in 2000 showed minimal chronic hepatitis with bridging fibrosis (Fig. 2C). The HAI categories I, II, III, and IV were scored 1, 1, 1, and 3, respectively.

At laparoscopy in 1979, patient 3 showed slight elevation of serum ALT activity (62 U/L) and had low levels of serum albumin (2.8 g/dL, normal value 3.8–5.3 g/dL), cholinesterase activity (64 U/L, normal value 97–249 U/L), and platelet count ($9.6 \times 10^4$/µL, normal value $13–35 \times 10^4$/µL). The liver biopsy specimen obtained at laparoscopy showed the HAI scores of categories I, II, III, and IV to be 3, 3, 3, and 4, respectively. Thus, this patient had moderate chronic hepatitis with cirrhosis (Fig. 2D). HCV RNA RT-PCR carried out on a stored serum sample revealed that he was already nonviremic at this point. During a 19-year follow-up period after laparoscopy, normalization was observed for serum ALT activity, albumin levels, cholinesterase activity, and platelet count, and the second liver biopsy in 1998 disclosed remarkable amelioration of the liver histology (Fig. 2E). The HAI scores after the follow-up were 0 for category I, 1 for category II, 1 for category III, and 3 for category IV. Only minimal chronic hepatitis was seen, and fibrosis was diminished significantly although bridging fibrosis still remained.

These long-term follow-up studies demonstrated that the stage of liver fibrosis as well as the grade of liver inflammation can improve in nonviremic HCV-seropositive chronic liver disease cases. A decrease in HCV antibody levels to multiple RIBA-3 antigens was observed during follow-up in each case, thus indicating recovery from previous HCV infections. Quantitative analysis of HCV core antibody revealed further reductions of HCV core antibody titers in two patients examined (patients 2 and 3). In patients 1 and 2, HCV RNA was measured using liver biopsy specimens obtained at the end of follow-up in 2000. Patient 1 tested negative for both positive and negative HCV RNA strands. However, patient 2 had the positive HCV RNA strand in the liver while the negative HCV RNA strand was not found. The three patients studied were persistently negative for serum HBsAg with anti-HBc and/or anti-HBs in patients 2 and 3. Each patient did not have serum HBV DNA by PCR during follow-up. At the end of follow-up, HBV DNA was also absent from the liver of Patients 1 and 2.

Liver biopsy specimens were also obtained from six patients (patients 4–9) who were also positive for HCV antibody by RIBA-3 but negative for serum HCV RNA (Table I). In contrast with patients 1–3, these six patients had no history of chronic liver disease. Serum ALT activity was normal in four patients and near-normal in patients 5 and 9. A low grade of liver inflammation was seen in all but patient 8. Liver histology showed persistent lobular necrosis and inflammation in four patients, portal inflammation in four, and piecemeal necrosis in one. Each category of necroinflammation was scored 1 for these cases except that steatosis was observed in patient 5 and his HAI score for category II was 4. No fibrosis was found in all but patient 4. Liver

fibrosis was scored 1 in patient 4. None of the six patients had the positive or negative HCV RNA strand in the liver. The six patients were all negative for serum HBsAg with anti-HBc and/or anti-HBs in patients 5 and 7. They were all negative for serum HBV DNA and liver HBV DNA.

## DISCUSSION

A considerable proportion of HCV antibody-positive individuals not treated with IFN are negative for serum HCV RNA. Recently, it has been demonstrated that spontaneous HCV clearance from the serum occurs in an unexpectedly high percentage of some populations [Vogt et al., 1999; Beld et al., 1999]. If serum HCV RNA is undetectable by RT-PCR at the end of IFN therapy and again 6 months after treatment, this has been taken to indicate HCV eradication by IFN, and patients with such sustained HCV RNA clearance from sera have been shown to continue to be liver HCV RNA-negative [Marcellin et al., 1997; Lau et al., 1998] accompanied by

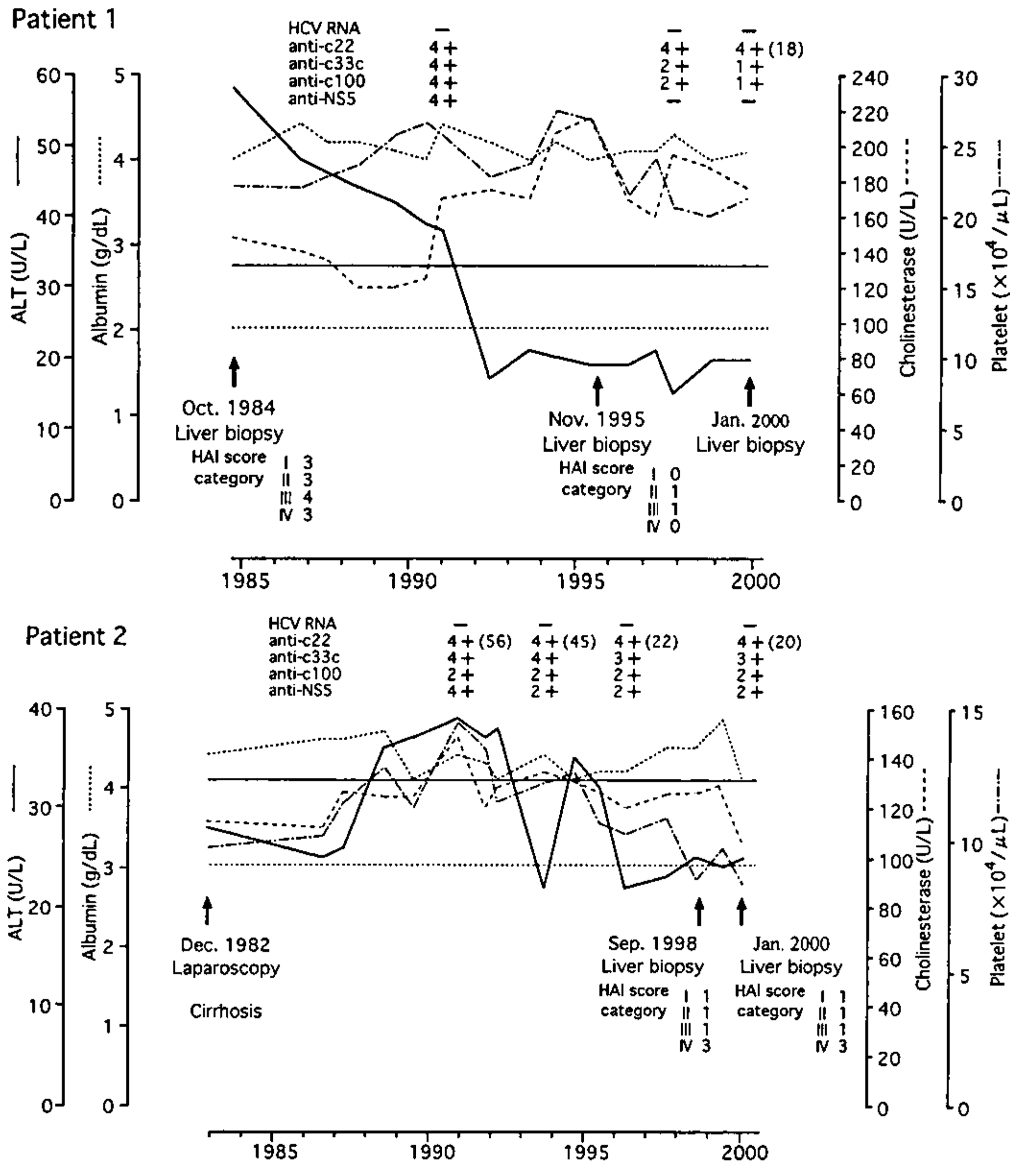


Fig. 1. Clinical, virological, and histological courses of three nonviremic HCV-seropositive patients without IFN therapy (patients 1, 2, and 3). All patients had undergone diagnostic laparoscopy for chronic non-A, non-B liver disease. After the discovery of HCV, they were found to be positive for HCV antibody but negative for serum HCV RNA and enrolled in this study. Solid and dashed horizontal lines represent the upper normal limit of ALT (33 U/L) and the lower normal limit of cholinesterase (97 U/L), respectively. Absolute titers of HCV core (c22) antibody are shown in parentheses.

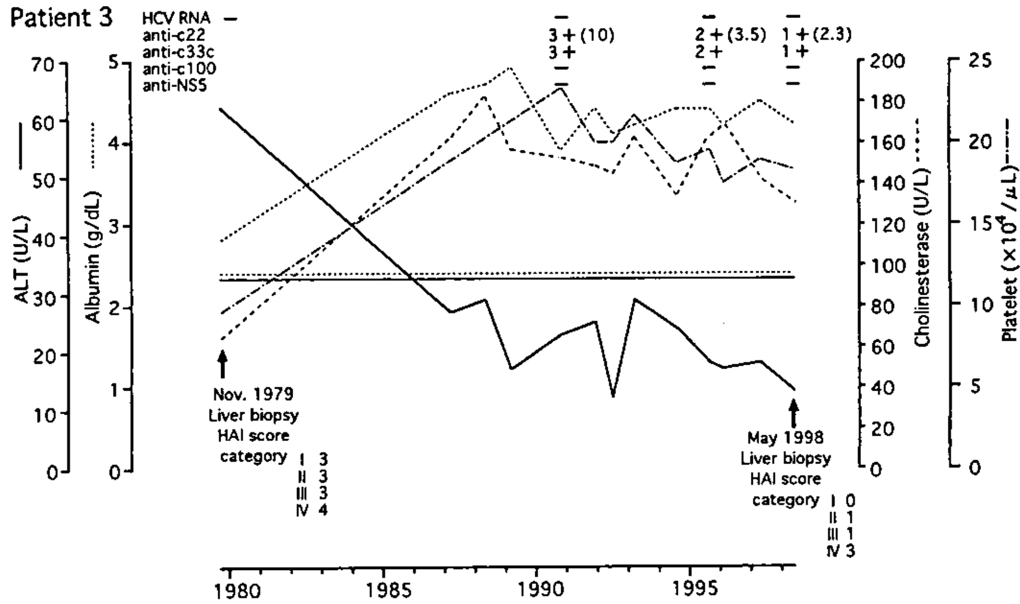


Fig. 1. (*Continued*)

sustained reductions of HCV antibody levels [Saracco et al., 1993;Yuki et al., 1993a,b; Diodati et al., 1994] and continuous improvement in histological findings [Marcellin et al., 1997; Lau et al., 1998]. In contrast, the clinical relevance of spontaneous serum HCV RNA clearance in HCV antibody-positive patients has not been well documented, and controversy remains.

The present study demonstrated that HCV antibody-positive but serum HCV RNA-negative patients without IFN therapy were likely to be negative for HCV

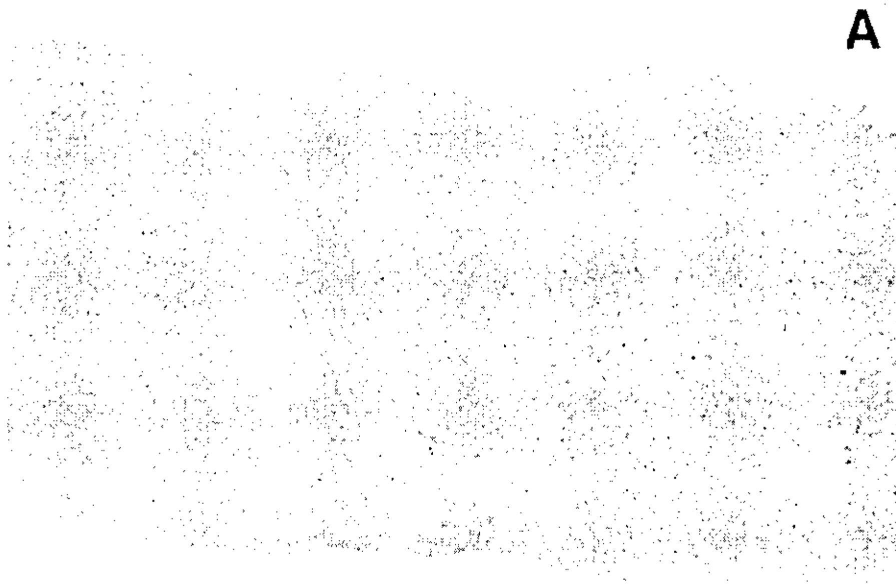


Fig. 2. Histological alterations in liver biopsy specimens years after HCV clearance from sera in the natural course. For the diagnosis of chronic non-A, non-B liver disease, laparoscopy was performed on patient 1 in 1984, patient 2 in 1982 (liver histology not available with laparoscopic diagnosis of established cirrhosis), and patient 3 in 1979. After serological assays for HCV were established in 1990, they remained positive for HCV antibody without viremia. Ultrasound-guided liver biopsies were performed on patient 1 in 1995, patient 2 in 2000, and patient 3 in 1998. (A) Patient 1 in Oct. 1984; (B) patient 1 in Nov. 1995; (C) patient 2 in Jan. 2000; (D) patient 3 in Nov. 1979; (E) patient 3 in May 1998. Paraffin sections stained with hematoxylin-eosin. (Original magnification ×16.)

nonstructural antibodies and have low titers of HCV core antibody. In addition, when nonviremic HCV-seropositive states of the three chronic liver disease patients were monitored over 8–9 years, reductions of HCV core and nonstructural antibodies occurred. Thus, the data obtained suggest that HCV antibody profiles of our patients were consistent with those observed years after successful IFN therapy and were indicative serologically of HCV eradication although further studies are necessary to confirm these observations. In the current study, the nonviremic HCV-seropositive patients were tested further for occult HCV infection in the liver. HCV RNA was not found in the liver biopsy specimens from any of the six patients who had no history of chronic liver diseases and were considered to have cleared HCV in the early phase of infection. On the other hand, persistence of the HCV genome was found in one of the two chronic liver disease patients examined who had been clear serologically of HCV for many years. Previous reports have suggested that HCV RNA can be detected in the

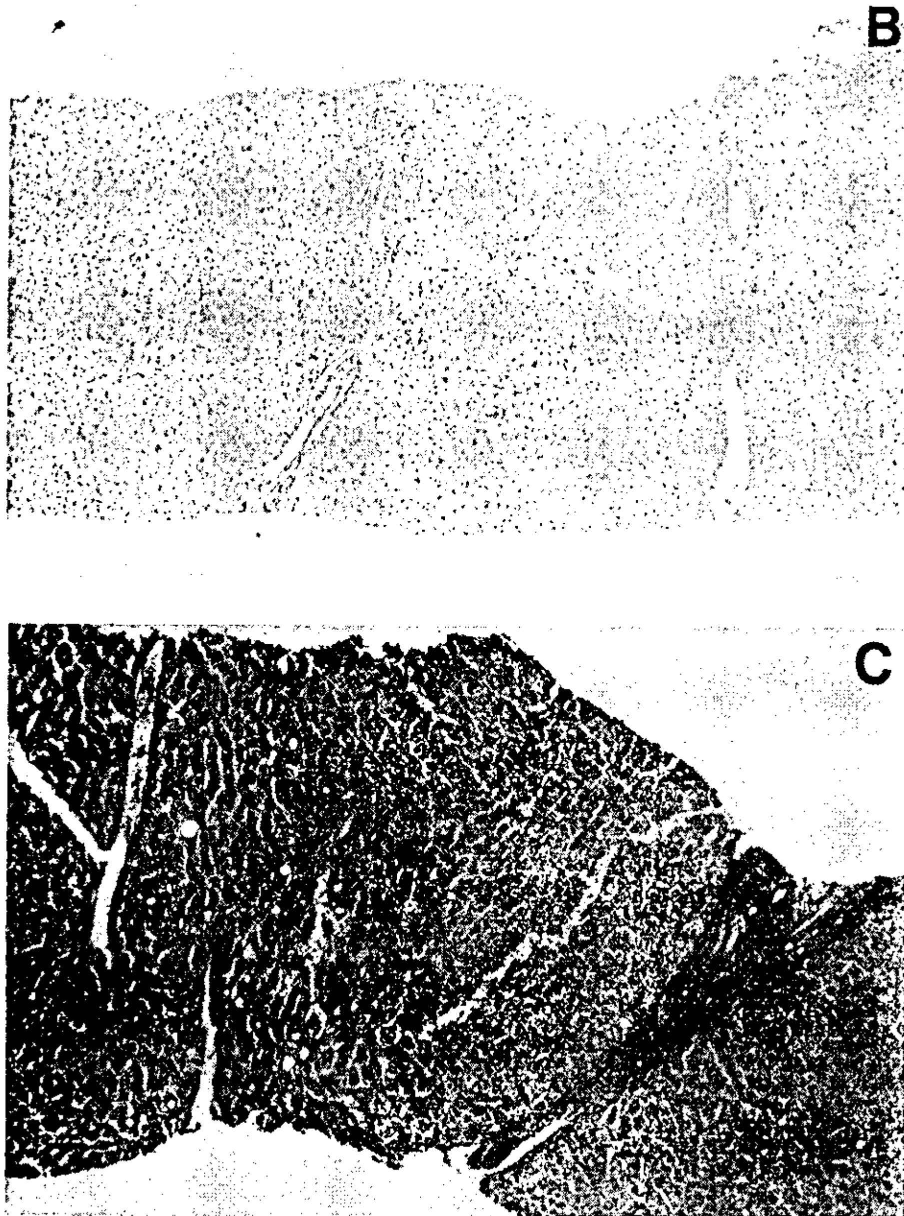


Fig. 2. (*Continued*)

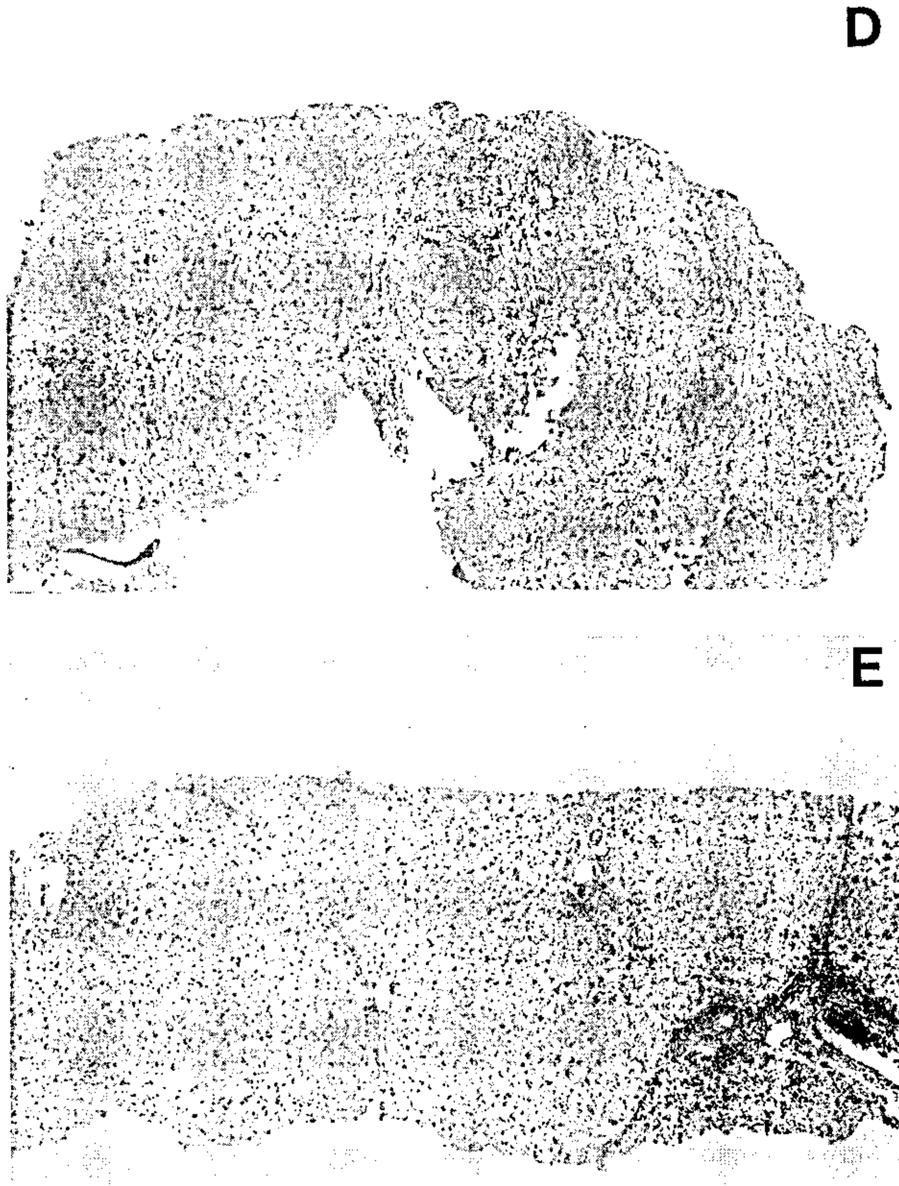


Fig. 2. (*Continued*)

liver, which generally harbors higher viral loads than the serum, in HCV antibody-positive but nonviremic patients without IFN therapy [Haydon et al., 1998; Dries et al., 1999]. In these cross-sectional studies, occult HCV infection was also shown to be associated with the presence of chronic liver disease on liver biopsy. Taken together, these observations raise the possibility that spontaneous serum HCV RNA clearance does not necessarily imply cure of HCV infection in chronic liver disease patients and the results of HCV RNA PCR for serum samples may not always serve as a reliable marker for HCV eradication. This presents a striking contrast to complete HCV eradication from the liver after successful IFN therapy for chronic hepatitis C. However, further studies are necessary to establish the range of such serologically undetectable occult HCV infections in chronic liver disease patients. Studying the HCV-specific cellular immune response may shed light on this issue. At present, what needs to be clarified is whether the prognosis of chronic liver diseases is really good once there has been spontaneous clearance of HCV RNA from the serum of HCV antibody-positive patients.

TABLE I. Clinical, Virological, and Histological Features of Nonviremic HCV-Seropositive Patients Without IFN Therapy*

| Patient no. | Date of liver biopsy | Age (yr) | Sex | Previous blood transfusion | History of liver disease | Serum ALT (U/L)[a] | Serum HCV RNA | Antibodies in RIBA-3 | | | | HCV core antibody titer (unit) | Liver histology[b] | | | | Liver tissue | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | anti-c22 | anti-c33c | anti-c100 | anti-NS5 | | I | II | III | IV | HCV RNA | HBV DNA |
| 1 | Jan. 2000 | 71 | F | — | Non-A, non-B CLD | 20 | — | 4+ | 1+ | 1+ | — | 18 | 0 | 1 | 1 | 0 | — | — |
| 2 | Jan. 2000 | 70 | M | — | Non-A, non-B CLD | 25 | — | 4+ | 3+ | 2+ | 2+ | 20 | 1 | 1 | 1 | 3 | + | — |
| 3 | May 1998 | 69 | M | — | Non-A, non-B CLD | 17 | — | 1+ | 1+ | — | — | 2.3 | 0 | 1 | 1 | 3 | NT | NT |
| 4 | Jul. 1999 | 51 | F | — | Acute hepatitis C | 11 | — | 4+ | 4+ | 4+ | — | 24 | 0 | 0 | 1 | 1 | — | — |
| 5 | Sep. 1999 | 63 | M | 46 yr before | Fatty liver | 50 | — | 4+ | 3+ | 1+ | — | 30 | 0 | 4 | 1 | 0 | — | — |
| 6 | Sep. 1999 | 52 | F | — | — | 9 | — | 4+ | 2+ | — | — | 7.2 | 1 | 1 | 1 | 0 | — | — |
| 7 | Oct. 1999 | 61 | F | — | — | 19 | — | 1+ | 4+ | — | — | ≤0.5 | 0 | 1 | 1 | 0 | — | — |
| 8 | Nov. 1999 | 70 | M | — | — | 16 | — | 3+ | — | — | — | 4.2 | 0 | 0 | 0 | 0 | — | — |
| 9 | Nov. 1999 | 62 | M | 49 yr before | — | 50 | — | 4+ | 1+ | — | — | 4.6 | 0 | 1 | 0 | 0 | — | — |

*CLD, chronic liver disease; NT, not tested. In patient 1, the liver biopsy specimen was subjected to only virological tests, and the histological data correspond to those obtained at the former liver biopsy in the long nonviremic HCV-seropositive period.
[a]Upper normal limit of ALT = 33 U/L.
[b]Four categories were scored: I, piecemeal necrosis; II, lobular necrosis and inflammation; III, portal inflammation; and IV, fibrosis.
All dashes indicate "negative."

In the current study, patient 2 with occult HCV infection also showed reductions of HCV antibody levels to core and nonstructural proteins. At the end of the follow-up, only the positive HCV RNA strand was found in the liver; no negative-strand RNA-replicative intermediate was found, thus indicating the absence of HCV replication. This patient also showed amelioration of liver histology. The previous laparoscopy had shown established cirrhosis with an apparently nodular liver surface. However, no histological feature of cirrhosis was seen when two liver biopsies were carried out at 16 and 17 years after laparoscopy. Only bridging fibrosis remained. Thus, it seems that occult HCV infection in the liver was accompanied by very low levels of replication and was well compartmentalized to restrict exposure to the host immune system and to exert little virulence. During the long-term follow-up of nonviremic HCV-seropositive states, regression of the liver fibrosis stage and improvement of the necroinflammatory grade were also evident for the other two chronic liver disease patients (patients 1 and 3). Complete loss of bridging liver fibrosis was observed in patient 1,and the histological features of liver cirrhosis had disappeared in patient 3. As for the persistence of occult HCV infection, patient 1 did not have detectable levels of HCV RNA in the liver, and patient 3 could not be tested for liver HCV RNA. Although further studies with more patients are necessary, the data obtained suggest that circulating HCV clearance in patients with chronic liver disease confers favorable liver histological outcomes even if the patients have cirrhosis. Occult HCV infection, if any, may be brought under full control by the host immune system with abrogation of further liver damage.

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# 慢性C型肝炎ウイルス感染患者に対するインターフェロン治療の血清KL-6値に及ぼす影響

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水野　重樹・山口　敏紀・小林　賢惣
福原　達磨

## I．緒　　言

慢性C型肝炎ウイルス（HCV）感染に対するインターフェロン（IFN）治療は広く行われ，その効果は確立されている[1),2)]。しかし，IFNの長期投与に伴う副作用も多く報告され，その中の間質性肺炎は重篤な副作用の一つであり[3)]，その早期発見と治療が必要である。

KL-6はMUC1に属するシアン化糖鎖抗原であり，肺ではII型肺胞上皮細胞，気管支腺細胞などで発現が認められ，抗KL-6抗体が認識する抗原KL-6が間質性肺炎患者の血清中で高率に異常高値を呈することが示されている[4)]。

本研究で，われわれは慢性HCV感染患者に対するIFN治療の血清KL-6に対する影響を検討し，IFN治療患者で血清KL-6測定の有用性を認めたので報告する。

## II．対象と方法

対象は2000年7月から2001年9月まで国立病院呉医療センターで慢性HCV感染に対してIFN治療が行われた42例（男性20例，女性22例）で，平均年齢は53.2±9.8歳（平均値±標準偏差）であった。IFN治療前の血清HCV RNAは全例陽性であり，HCV RNA量は＜100 K copies/mLが11例，＜300 K copies/mLが6例，≧300 K copies/mLが25例であった。使用されたIFNの種類は天然型IFNαが36例，組み換え型IFNα-2bが6例で，いずれも2週間連日，その後，週3回の合計24週間投与され，総投与量は583.3 ± 166.1MUであった。肝の線維化は新犬山分類[5)]で$F_0$ 1例，$F_1$ 19例，$F_2$ 10例，$F_3$ 11例，$F_4$ 1例であった。

IFN治療前，治療開始後1カ月毎に血清KL-6，一般的肝機能検査，血球数を測定し，IFN治療前，治療開始1カ月後，3カ月後，治療終了時，治療終了後6カ月目に血清HCV RNAを測定した。

血清KL-6はECLIAで測定し，血清HCV RNAはRT-PCRで測定し，治療終了後6カ月目の血清HCV RNA値が検出不能であった症例をIFN反応者と判定した。

統計学的解析はWilcoxon signed-ranks testで有意性を解析した。

## III．結　　果

42例の中でIFN治療に対する反応者は10例であり，他はIFN治療中に血清HCV RNAが検出不能となったが，IFN治療中止後，再陽性化した一過性反応者あるいは全く反応しなかった非反応者であった。

血清KL-6値はIFN治療前259.9±176.5 U/mL（n＝42），IFN治療開始1カ月後251.0±81.6 U/mL（n＝21），2カ月後300.6±228.8 U/mL（n＝30），3カ月後316.3±165.3 U/mL（n＝38），4カ月後324.0±195.7 U/mL（n＝36），5カ月後311.2±128.4 U/mL（n＝36），治療終了時336.7±143.2 U/mL（n＝33），治療後1カ月目316.9±206.4 U/mL（n＝25）であった（図1）。IFN治療中にKL-6異常高値が2例に認められ，これら2症例を提示する。

症例1は56歳の男性で，治療前の肝組織の線維化は$F_2$であり，治療前血清HCV RNA量は1,200.0 K copies/mLであった。2000年10月30日に天然型IFNα 1,000万単位，2週間連日，その後，週3回合計24週の予定でIFN治療を開始した。治療前の検査値は血清KL-6が1,270 U/mL，LDHが403 IU/L，白血球数が6,300/$mm^3$であった。血清KL-6はIFN治療開始後50日目に1,450 U/mL，71日目に1,190 U/mLを示し，その後，全身の難治性の湿疹のため不眠に陥り，IFN治療を2001年1月9日に中止した。IFN中止後38日目のKL-6値は1,130 U/mLであった。IFN治療中の血清LDHは治療開始後3日目の439 IU/Lがピーク値であり，白血球数は治療開始後71日目の7,700/$mm^3$がピーク値であった（図2）。IFN治療前の2000年10月

Eiichi Takezaki, Sinzoh Murakami, Toshitaka Tuda, Shigeki Mizuno, Toshiki Yamaguchi, Kensoh Kobayashi, Tatsuma Fukuhara: Influence of interferon therapy on serum KL-6 values in patients with chronic hepatitis C virus infection. Department of Gastroenterology, National Kure Medical Center.
国立病院呉医療センター消化器科

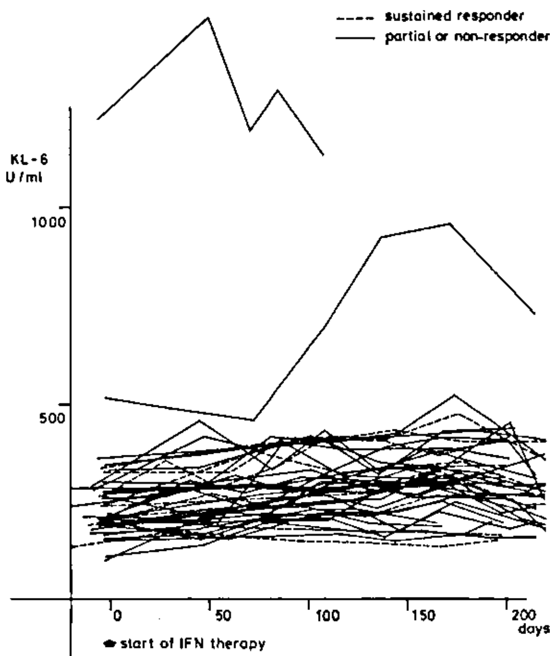


図1　インターフェロン治療前，中，後の血清KL-6値の推移

（点線はインターフェロン持続反応者，実線は一過性反応者と非反応者を示す）

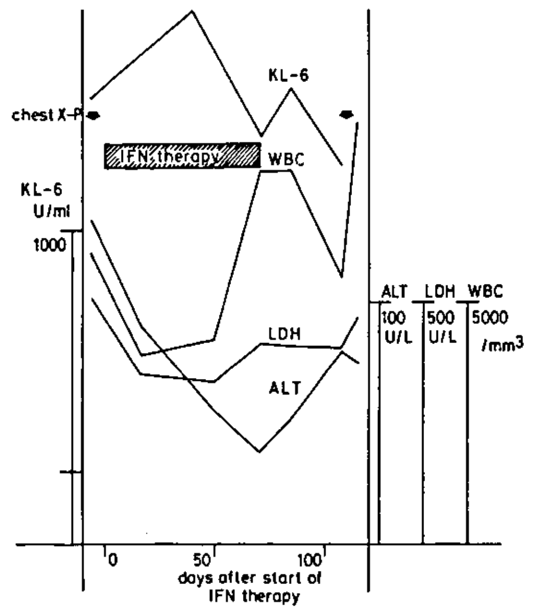


図2　症例1のインターフェロン治療前，中，後の血清KL-6，LDH，ALT値と白血球数の推移

24日に撮影された胸部X-P写真と治療中止時の2001年1月9日に撮影された胸部X-P写真に異常所見は認められなかった（図3）。慢性C型肝炎のIFN治療（総投与量350 MU）に対する反応は治療中に血清HCV RNAが検出不能となったが，治療後，再陽性化し，一過性反応であった。

症例2は60歳の女性で，治療前の肝組織の線維化は$F_3$であり，治療前血清HCV RNA量は1,070.0 K copies/mLであった。2000年7月21日に天然型IFNα 600万単位，2週間連日，その後，週3回合計24週間の予定でIFN治療を開始し，2001年1月21日にIFN治療を診療した。治療前の検査値は血清KL-6が515 U/mL，LDHが382 IU/L，白血球数が3,900/mm$^3$であった。血清KL-6はIFN治療開始後172日目の957

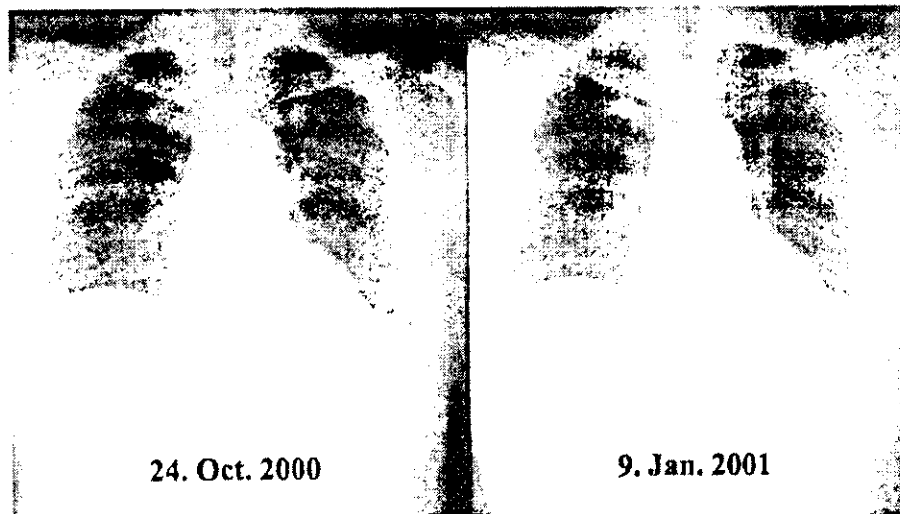


図3　症例1の2000年10月24日（左）と2001年1月9日（右）に撮影された胸部X-P写真

U/Lがピーク値であり，血清LDHはIFN治療開始後137日目の466 IU/Lがピーク値であり，白血球数はIFN治療開始後46日目の4,200/$mm^3$がピーク値であった（図4）。IFN治療前の2000年7月17日に撮影された胸部X-P写真と治療137日目の2001年12月5日に撮影された胸部X-P写真に異常所見は認められなかった（図5）。慢性C型肝炎のIFN治療（総投与量504 MU）に対する反応は，症例1と同様に一過性反応であった。

表1に血清KL-6値の各月毎からみた統計学的な相関関係を示す。血清KL-6値はIFN治療前値と比較すると治療開始3カ月後から6カ月後まで有意な高値を示し，さらに，7カ月後（治療終了後1カ月目）との間にも有意差が認められたが，6カ月後（治療終了時）と比較すると有意な低下がみられた。前後する月との比較でIFN治療後の血清KL-6値の動向をみると，KL-6はIFN治療開始後徐々に上昇し，治療開始3カ月後にピーク値に達し，その後6カ月後までピーク値を維持し，治療終了後（治療7カ月後）に治療前値に戻る傾向を示した。

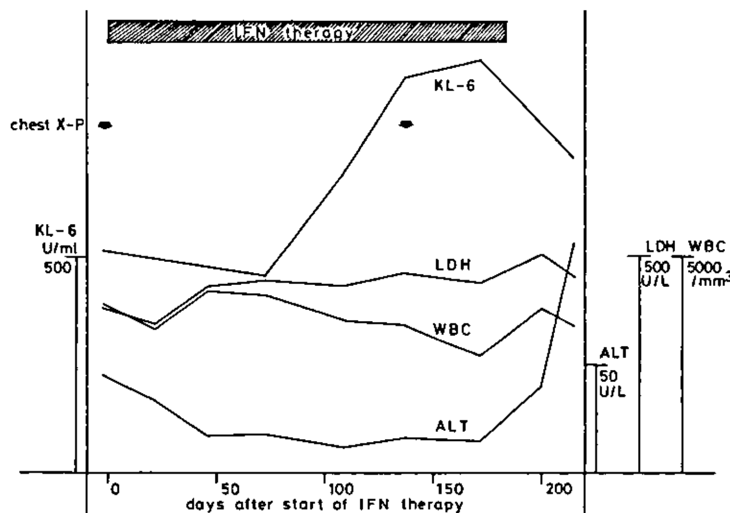


図4　症例2のインターフェロン治療前，中，後の血清KL-6，LDH，ALT値と白血球数の推移

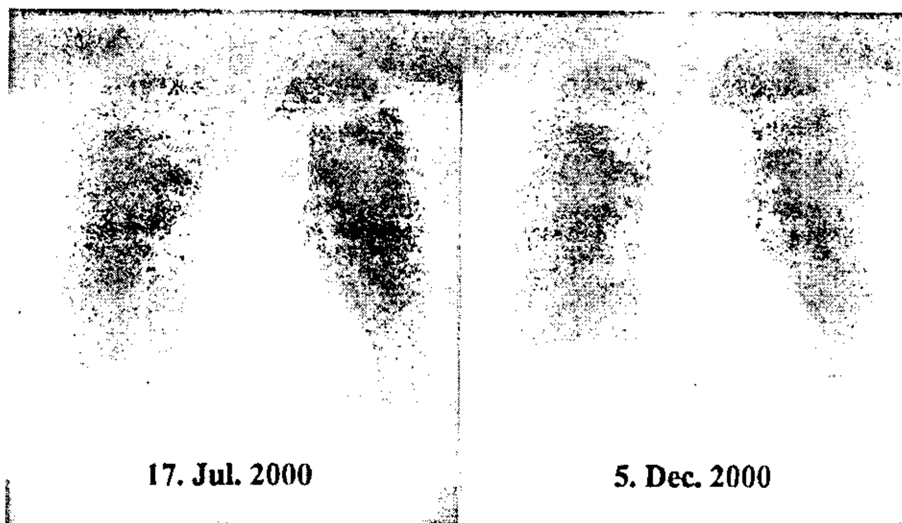


図5　症例2の2000年7月17日（左）と2001年12月5日（右）に撮影された胸部X-P写真

表1　インターフェロン治療前，中，後の血清 KL-6 値の比較検査
（表中の数字は有意差検定の P 値を示す）

| months | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 0 | 0.0032 | 0.0003 | <0.0001 | <0.0001 | <0.0001 | 0.0143 | |
| 1 | | 0.4204 | 0.0035 | 0.0313 | 0.1222 | 0.0235 | 0.6155 |
| 2 | | | 0.0404 | 0.0448 | 0.0018 | 0.0156 | 0.2954 |
| 3 | | | | 0.6583 | 0.2689 | 0.0710 | 0.0111 |
| 4 | | | | | 0.1222 | 0.0235 | 0.0143 |
| 5 | | | | | | 0.0585 | 0.0111 |
| 6 | | | | | | | 0.0017 |

## Ⅳ．考　　察

KL-6 は高分子量ムチン様の肺腺癌関連抗原の一つとして発見され[6]，ムチンの MCU1 に属する肺細胞抗原のクラスター 9 に分類している[7]。正常肺で KL-6 分子はⅡ型肺胞上皮細胞，呼吸細気管支上皮細胞，気管支腺漿液細胞などに発現され[8),9]，肺に広範なリモデリングを来す各種の間質性肺炎で高い陽性率が報告されている[10]。間質性肺炎で血清 KL-6 が上昇する機序について，胞隔炎によって空気血液関門が傷害され，KL-6 のような大分子が気道被膜液中から血液内へ流入すること，また，空気血液関門の傷害が肺胞領域，間質と気腔の関門の傷害を伴い，この傷害個所に KL-6 産生再生肺上皮細胞が存在することによる気道被覆液中の KL-6 濃度が上昇することが推測されている[11]。

慢性 C 型肝炎の IFN 治療による間質性肺炎症例の約 2/3 は漢方薬の小柴胡湯との併用例であるが，IFN 単独治療による間質性肺炎発症は約 0.2% 前後と報告されている[12]。IFN による間質性肺炎発生機序は不明であるが，IFN によるアレルギー反応，IFN により誘導される種々のサイトカイン（IL-2，IL-2 レセプターの関与など）が免疫反応を誘発することが考えられ[11]，さらに，IFN によって間質性肺炎が発症する素因として，高齢者，肺野の間質陰影の増強している患者，小柴胡湯併用者が指摘されている。

間質性肺炎は IFN 治療の後期（IFN 治療開始後 2 カ月以降）に発症する副作用であることが知られている。われわれが，今回，対象とした 42 例の中に小柴胡湯併用症例はなく，また，臨床的に間質性肺炎と診断した症例もなかったが，IFN 治療中の血清 KL-6 値が治療開始後徐々に上昇し，3 カ月目にピーク値に達し，治療の 6 カ月目までこのピーク値が持続したことは，間質性肺炎が IFN 治療の後期に発症する副作用であることを支持する証拠であると考えられる。

今回の 42 例の中で 2 例に血清 KL-6 異常値が認められた。1 例は IFN 治療前から KL-6 異常値を示し，1 例は IFN 治療中に異常値を示した。熊田[13]は，慢性 C 型肝炎の 5% が KL-6 陽性であり，間質性肺炎合併慢性 HCV 感染患者の 95% が KL-6 陽性であることを示し，さらに，これら KL-6 陽性患者の中には，1〜2 年前に遡った検査でも KL-6 が陽性であった症例が存在することを報告している。われわれの 2 例は現在まで間質性肺炎を発症していないが，今後，綿密な観察が必要と考えられる。最近，初回治療に反応しなかった慢性 HCV 感染患者の IFN 単独あるいは他の薬剤との併用による再治療が広く行われているが，IFN 初回治療中に KL-6 異常値を示した症例は，IFN を含む薬剤による再治療時にはさらに綿密な観察が必要であると考えられる。

## Ⅴ．結　　語

IFN 治療中の慢性 HCV 感染患者 42 例の血清 KL-6 値の変動と肺線維症の関連を検討した。血清 KL-6 値は IFN 治療開始後徐々に上昇し，IFN 治療の約 3 カ月目にピーク値に達し，IFN 治療終了後低下した。IFN 治療中に 2 例が血清 KL-6 異常高値を示したが，臨床的に肺線維症は認められなかった。現在，慢性 HCV 感染患者の IFN 再治療が広く行われているが，IFN 治療中に血清 KL-6 異常高値を示した経験のある患者は厳密な観察が必要であると考えられた。

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（受付　2002 – 11 – 29）

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## Response to Drs. Jacobs *et al.*

TO THE EDITOR: We appreciate the interest by Jacobs *et al.* (1) regarding our article (2) and thank them for their comments. Both points raised by them could have the effect of improving the cost-effectiveness ratio of vaccination against acute hepatitis A viral infection.

We acknowledge that underreporting of cases of acute hepatitis A may occur as many infections may be asymptomatic or mildly symptomatic (3). However, for our base-case analysis, we considered it appropriate to use the reported rate to limit the number of assumptions. To account for the possibility of underreporting, we performed sensitivity analyses of the effects of varying the incidence of hepatitis A on the incremental cost-effectiveness ratio of the vaccination strategies (see Table 5 in our article [2]). Specifically, if the incidence rate is 50% over the reported incidence rate, the incremental cost-effectiveness ratio would improve from $51,000 per quality-adjusted life year (QALY) to $31,000 per QALY.

We understand the concern of Jacobs *et al.* (1) about the controversial nature of the appropriate outcome measure to use in cost-effectiveness analyses. We elected to use QALYs given that this measure incorporates both quality of life and survival and permits comparisons with prior published cost-utility analyses (4, 5). When we reanalyzed our data using years of life saved as our outcome measure, we obtained an incremental cost-effectiveness ratio of $46,000 per year of life saved. We fully agree with Jacobs *et al.* (1) about the value of human life but disagree that using QALYs in our analysis in any way supports withholding preventive interventions in any patient group.

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## Intraspousal Transmission of Hepatitis C Virus Occurring After 42 Years of Marriage: No Relation to HCV RNA Titers

TO THE EDITOR: We report a case of acute hepatitis C transmitted most probably by sexual intercourse from a 72-yr-old man with chronic hepatitis C to his 63-yr-old wife after a 42-yr marriage. We had also observed monthly changes of hepatitis C virus (HCV) RNA titers of the husband for 1 yr before transmission to his wife.

Blood transfusion is considered the main route of transmission of HCV; however, other mode of transmissions have also been reported, such as *i.v.* drug abuse, tattoos, and from mother to child. A few cases of transmission by sexual intercourse between husband and wife have also been reported by analyzing mainly seroepidemiologically anti-HCV antibody and/or genotype (1–7). So far, only six cases of acute HCV infection transmitted from a sexual partner were confirmed by nucleotide sequence analysis (3, 5, 7–10). Among them, only two cases were intraspousal transmission, which occurred after a long period of marriage (3, 5).

A 63-yr-old woman had been followed-up from 1991 to 1995 at our hospital because of hepatic dysfunction of unknown etiology. The liver enzyme test had normalized without therapy since May, 1992. Anti-HCV was negative in 1991, 1993, and 1995, and HCV RNA was also negative in 1993 and 1995. She lastly visited our hospital in September 1995. Thereafter, her data of health checkups

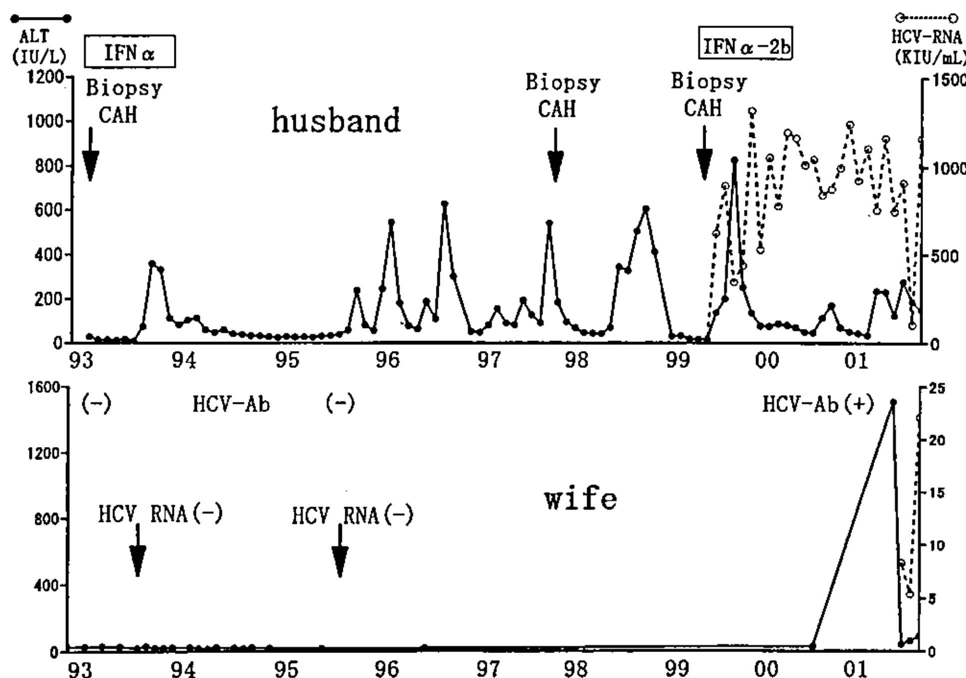


**Figure 1.** Time course of husband and wife. CAH = chronic active hepatitis; IFN = interferon.

showed normal liver enzyme levels including ALT, AST, total bilirubin, and Zinc sulfate turbidity test in October 1996 and July 2000. She suddenly complained of general fatigue, poor appetite, and jaundice on April 13, 2001, and acute hepatitis C was diagnosed at the nearby hospital (negative for HBsAg, positive for anti-HCV, AST 1050 IU/L, ALT 1510 IU/L, total bilirubin 1.5 mg/dl). Those abnormal liver enzymes normalized at the fourth week of admission, and she was referred to our hospital in May 2001 (ALT 26 IU/L, AST 27 IU/L). HCV genotype was 1b. HCV RNA titers in May, June, and July 2001 after development of acute hepatitis C were 8.3, 5.2, and 23 KIU/ml, respectively (Fig. 1). A needle biopsy specimen of the liver taken in September 2001 showed no fibrosis, only mild necroinflammation of the parenchyma, and a slight lymphocytic reaction in the portal area, which suggested acute hepatitis without the evidence of chronic inflammation.

On the other hand, her 72-yr-old husband had been followed-up because of chronic hepatitis C (genotype 1b) since 1988 at our hospital. He had a past history of blood transfusion from on operation for pulmonary tuberculosis in 1957. He was first diagnosed with abnormal liver function in 1983. Interferon-$\alpha$ was administered for 6 months in 1993 and 1998, totaling 780 MU and 460 MU each time, without response, and thereafter ALT titers had remained in the abnormal range between 43 to 483 IU/L. HCV RNA titers measured monthly had fluctuated in the range between 11 and 25 Meq/ml (branched DNA probe) from January 1994 to April 1997, between 396.3 and 2100 KC/ml from May 1997 to November 2, 1998 (data not shown in Fig. 1), had become less than 1.0 KIU/ml from November 25, 1998, to May 1999 after starting interferon-$\alpha$ 2b, and again had fluctuated in the range between 345 and 1240 KIU/ml since June 1999 without an apparent increase within 6 months of onset of acute hepatitis C in his wife. Liver biopsy specimens taken in 1993, 1997, and 1998 all showed chronic active hepatitis (Fig. 1).

Nucleotide sequence of the HCV core and estrone (E1) genomes were determined by using serum from the wife collected on May 11, 2001, and serum from the husband collected on May 31, 2001. The sequence variation of the core and E1 regions were compared using clones of other genotypes: HPCCGAA(1a), HPCJTB(1b), HPCPOLP(2a), HPCJ8G(2b), E-b1(3a), HCV-TR(3b), CR4N1(4), SA-K3 (5a), and HK2(6a) for the core region and HPCCGAA(1a), HPCJTB-E1(1b), HPCPOLP(2a), HPCJ8G(2b), AF046866 (3a), L39314(4a), SA1-5a, SA13-5a, and HK2-6a for the E1 region, respectively. As a consequence, the nucleotide sequence of the HCVs isolated from the wife showed 98.1% homology with her husband in the core region but low homology with clones from other sources (79.7–91.6%), and 96.3% homology with her husband in the E1 region but low homology with clones from other sources (45.9–69.2%).

Akahane *et al.* described that the intraspousal transmission-rate of HCV increases with a longer duration of marriage (4). Okushin *et al.* (1) analyzed 50 spouses of chronic hepatitis C patients, resulting in anti-HCV-positive spouses

who were over 40 yr old and married for over 20 yr, and 57.7% of anti-HCV-positive spouses who were over 50 yr old and married for over 27 yr. This suggests a longer incubation period of sexual transmission of HCV. As to the relation between HCV transmission and HCV RNA titers, there is a report that the mean titers of HCV RNA were slightly higher in men who appeared to transmit HCV to their female partners compared with men who failed to transmit HCV to their female partners, although there was some overlap in the range of values (11). However, in our case, the HCV RNA titers of the husband measured monthly fluctuated in the range between 345 and 1240 KIU/ml without an apparent increase before transmission of HCV to his wife.

The reason why sexual transmission of HCV occurred after a long-lasting marriage is unknown, despite regular sexual intercourse without extramarital intercourse, that is, once or twice a month for the last 10 yr. They did not have blood transfusion during the marriage. There was no evidence of *i.v.* drug use in their life until now. Both patients tested negative for human immunodeficiency virus. They used their own toothbrush and did not have razors in common use. They had not undergone dental treatment, any operation, or acupuncture at least in the past 5 yr. These evidences and higher homology of nucleotide sequence in the core and E1 region between the spouses strongly suggest the intraspousal sexual transmission. One possible explanation might be that immune competence against the invasion of the virus through injured skin and mucosa decreases with age.

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## *Helicobacter pylori* Infection Does Not Affect the Serum Level of Homocysteine

TO THE EDITOR: Hyperhomocysteinemia has been reported to cause vascular endothelial damage with resulting coronary arteriosclerosis (1). Malabsorption of vitamins caused by *Helicobacter pylori* (*H. pylori*) infection may affect the serum homocysteine concentration (2), and result in arteriosclerosis (3, 4). However, the findings of previous studies concerning the effect of *H. pylori* infection on homocysteine concentration are still conflicting (5–8). Recently, we have found that *H. pylori*-seropositive elderly Japanese individuals have a significantly lower serum high-density lipoprotein cholesterol concentration than *H. pylori*-seronegative ones (9), suggesting that long-term infection with *H. pylori* may have an effect on the development of arteriosclerosis and the onset of cardiovascular disease. However, there was no difference in high-density lipoprotein cholesterol level between *H. pylori*-positive and negative individuals under the age of 60 yr. Therefore, we investigated the serum homocysteine concentration in *H. pylori*-positive and negative individuals to clarify whether *H. pylori* infection affects homocysteine metabolism as a cause of arteriosclerosis in middle-aged individuals. The subjects were 45 healthy volunteers with *H. pylori* infection (mean age 39.8 yr, range 29–53 yr, 24 men), and 45 age-matched controls without infection (mean age 39.4 yr, range

Short Communication

# Molecular and serological characterization of sporadic acute hepatitis E in a Japanese patient infected with a genotype III hepatitis E virus in 1993

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Serum samples collected periodically from a 40-year-old Japanese woman who had not travelled abroad and who had contracted sporadic acute hepatitis E in 1993 were semi-quantitatively tested by enzyme immunoassay for IgM, IgA and IgG antibodies to hepatitis E virus (HEV). Anti-HEV IgM and IgA antibody levels were the highest (1 : 2400 dilution and 1 : 3400 dilution, respectively) on day 9 after the onset of hepatitis and then decreased rapidly in a parallel manner. Anti-HEV IgG antibody levels were the highest (1 : 17000 dilution) on day 145 and then decreased gradually but remained at high titres (1 : 2200 dilution) even 8·7 years after the onset of hepatitis. An HEV isolate, HE-JA10, recovered from the patient's serum at admission was closely related to a genotype III strain isolated in the United States (US1), with 92·2% identity over the full-length genome, and was most closely related to the JMY-Haw isolate of Japanese origin (95·4% identity).

Hepatitis E virus (HEV) is a major cause of epidemic and sporadic, enterically transmitted non-A, non-B hepatitis in many developing countries in Asia, Africa and Latin America (Purcell & Emerson, 2001a). Recently, there is growing consensus that (i) HEV-associated hepatitis also occurs among individuals in industrialized countries with no history of travel to areas endemic for HEV (Harrison, 1999; Purcell & Emerson, 2001a; Schlauder & Mushahwar, 2001) and (ii) HEV is a zoonotic virus, as suggested by the close genetic relationship between human and swine viruses (Erker *et al.*, 1999; Hsieh *et al.*, 1999; Meng *et al.*, 1997, 1998). The genome of HEV is a single-stranded, positive-sense RNA of approximately 7·2 kb and contains a short 5′ untranslated region (UTR), three open reading frames (ORFs 1–3) and a short 3′ UTR terminated by a poly(A) tract (Reyes *et al.*, 1990; Tam *et al.*, 1991). HEV sequences have been classified tentatively into four major genetic groups (genotypes I–IV) (Schlauder & Mushahwar, 2001). Worldwide, the majority of HEV infections are caused by genotype I, while only isolated cases of infection with HEV of genotype III or IV have been described in the United States, Europe, Argentina, Taiwan and China (Hsieh *et al.*, 1999; Kwo *et al.*, 1997; Pina *et al.*, 2000; Schlauder *et al.*, 1998, 1999, 2000; Wang *et al.*, 1999, 2000, 2001; Worm *et al.*, 2000; Zanetti *et al.*, 1999). In Japan, multiple HEV strains of genotype III or IV have been isolated from patients with acute hepatitis of non-ABC aetiology who had never been abroad (Takahashi *et al.*, 2001, 2002a, b; Mizuo *et al.*, 2002), and swine HEV strains of genotype III have been isolated from farm pigs in Japan (Okamoto *et al.*, 2001). These results indicate that heterogeneous HEV strains are circulating in Japan and that domestically infected hepatitis E occurs in Japan, where HEV infection had been considered to be non-endemic. However, the molecular and serological characteristics of sporadic acute hepatitis E in industrialized countries, including Japan, are not fully understood. Therefore, in the present study we tested serum samples that had been obtained periodically from a 40-year-old Japanese female who contracted sporadic acute hepatitis E in 1993 [patient 10 in our previous report (Mizuo *et al.*, 2002)] for the relative titres of anti-HEV IgM, IgA and IgG antibodies and for HEV RNA; the entire genomic sequence of the HEV strain isolated from the infected patient was determined also to define its genomic characteristics.

To detect anti-HEV IgG and IgM antibodies, ELISAs were performed using purified recombinant ORF2 protein of HEV genotype IV that had been expressed in the pupae

The entire nucleotide sequence of the HE-JA10 isolate reported herein has been assigned DDBJ/EMBL/GenBank accession no. AB089824.

of silkworm, as described previously (Mizuo *et al.*, 2002). For the anti-HEV IgA assay, peroxidase-labelled rabbit IgG $(Fab')_2$ against human IgA (Dako) was used in place of the enzyme-labelled anti-human IgG or IgM antibodies. The cut-off absorbance value (read at a wavelength of 450 nm) used for the anti-HEV IgG assay was 0·152 and that for the anti-HEV IgM assay was 0·353. The tentative cut-off absorbance value for the anti-HEV IgA assay was determined to be 0·350, according to the method described previously (Mizuo *et al.*, 2002). The relative titres of anti-HEV IgG, IgM or IgA antibodies were determined by end-point ELISA; i.e. the serum dilution that would give the absorbance value (measured at a wavelength of 450 nm) of each cut-off point was estimated by testing multiple dilutions of the serum.

To quantify HEV RNA, total RNA was extracted from 100 μl of serum or its dilutions, reverse-transcribed and then subjected to nested PCR with ORF2-specific primers, as described previously (Mizuo *et al.*, 2002). The highest dilution of serum ($10^N$) found positive was estimated and converted to the titre $ml^{-1}$ of serum.

To sequence the full length of the genome, a central 7 kb sequence of the HEV genome (HE-JA10) was divided into six overlapping sections and amplified by PCR. These six overlapping fragments were nt 43–1270 (1228 nt), 1238–2641 (1404 nt), 2623–3905 (1283 nt), 3899–5327 (1429 nt), 5273–6398 (1126 nt) and 6362–7145 (784 nt) (primer sequences excluded). The extreme 5′ end sequence (nt 1–70) was determined by a modified RACE technique, RNA ligase-mediated RACE (RLM-RACE), using the First Choice RLM-RACE kit (Ambion). Amplification of the extreme 3′ end sequence [nt 7119–7244, excluding the poly(A) tail] was attempted by RACE according to the method described previously (Okamoto *et al.*, 2001). Amplification products were sequenced on both strands either directly or after cloning into the pT7BlueT vector (Novagen) and sequence analysis was performed as described previously (Takahashi *et al.*, 2002b). Phylogenetic trees were constructed using the neighbour-joining method (Saitou & Nei, 1987) and final trees were obtained using the TREEVIEW program, version 1.6.6 (Page, 1996).

In September 1993, a 40-year-old woman visited an internal medicine clinic and presented with general malaise and nausea. Because she had elevated liver function tests [1019 IU alanine aminotransferase (ALT) $l^{-1}$ serum and 590 IU aspartate aminotransferase (AST) $l^{-1}$ serum], she was admitted to our hospital on day 6 from disease onset and followed for 281 days. The results of the initial tests were as follows: ALT, 541 IU $l^{-1}$; AST, 174 IU $l^{-1}$; total bilirubin, 2·4 mg $dl^{-1}$; and thymol turbidity test, 19·0 KU (normal, 0–4 KU). Liver pathology on biopsy specimens obtained on day 9 from disease onset showed typical findings of acute viral hepatitis, unaccompanied by morphological cholestasis. On admission, HEV RNA was detected at an endpoint dilution of 1:1000 and IgM, IgA and IgG classes of anti-HEV antibodies were detected, each with a relative titre of > 1:2000 dilution. The titre of HEV RNA decreased rapidly and HEV viraemia continued up through day 23. The anti-HEV IgM antibody level was the highest (1:2400 dilution) on day 9 and then decreased rapidly, in parallel with anti-HEV IgA antibody levels. The relative titre of anti-HEV IgG antibodies was highest on day 145 (1:17 000 dilution) and then gradually decreased but remained at high titres (1:2200 dilution) even 8·7 years after the onset of hepatitis (Table 1).

The HE-JA10 isolate had a genomic length of 7244 nt, excluding the poly(A) tract at the 3′ terminus, and possessed three major ORFs, similar to those reported for human and swine HEV isolates. ORFs 1–3 have a coding capacity of 1709 (nt 26–5152), 660 (nt 5190–7169) and 122 aa (nt 5152–5517), respectively. Comparison of the HE-JA10 genome against reported HEV genomes of genotypes I–IV, whose entire or nearly entire nucleotide sequences

**Table 1.** Laboratory parameters and relative titres of anti-HEV antibodies and HEV RNA in serum samples obtained periodically from the patient with HEV-associated hepatitis

The normal ranges of total bilirubin, ALT and AST are 0·2–1·0 mg $dl^{-1}$, 4–34 IU $l^{-1}$ and 9–31 IU $l^{-1}$, respectively. NT, Not tested.

| Days (years) after onset | Total bilirubin (mg $dl^{-1}$) | ALT (IU $l^{-1}$) | AST (IU $l^{-1}$) | Relative titre of anti-HEV assay | | | HEV RNA (PCR titre $ml^{-1}$) |
|---|---|---|---|---|---|---|---|
| | | | | IgG | IgM | IgA | |
| 3 | NT | 1019 | 590 | NT | NT | NT | NT |
| 6 | 2·4 | 541 | 174 | 1:6 700 | 1:2 100 | 1:2 600 | $10^3$ |
| 9 | 2·1 | 281 | 79 | 1:14 000 | 1:2 400 | 1:3 400 | $10^2$ |
| 23 | 0·8 | 39 | 25 | 1:13 000 | 1:1 400 | 1:2 100 | $10^1$ |
| 47 | 0·5 | 18 | 19 | 1:10 000 | 1:560 | 1:1 000 | – |
| 61 | 0·6 | 16 | 17 | 1:12 000 | 1:330 | 1:750 | – |
| 145 | 0·4 | 17 | 18 | 1:17 000 | 1:190 | 1:430 | – |
| 173 | 0·4 | 20 | 17 | 1:15 000 | 1:180 | 1:300 | – |
| 287 | 0·5 | 19 | 18 | 1:8 400 | 1:130 | 1:180 | – |
| 3186 (8·7) | 0·5 | 12 | 16 | 1:2 200 | <1:100 | <1:100 | – |

are known (see legend to Fig. 1), revealed that HE-JA10 was most closely related to JMY-Haw, with identities of 95·4, 95·2, 96·0 and 98·4 % in the nucleotide sequence of the full genome, ORF1, ORF2 and ORF3, respectively. The HE-JA10 isolate was closely related to a genotype III isolate in the United States (US1) with 92.2% identity over the full-length genome and only 73·5–75·6 % similar to the human and swine HEV isolates of genotypes I, II and IV. The phylogenetic tree constructed based on the full genomic sequence confirmed that HE-JA10 belongs to genotype III and is most closely related to JMY-Haw (data not shown). The 5′ UTR of HE-JA10 comprised 25 nt, with a sequence beginning GCAGACCAC, similar to the 5′ UTRs of the P1 strain of genotype I, the MEX-14 strain of genotype II, the swine strain of genotype III (swJ570) and the HE-JI4 strain of genotype IV, which had been determined by RLM-RACE (Fig. 1a), the presence of a cap structure being taken into consideration (Kabrane-Lazizi *et al.*, 1999). Therefore,

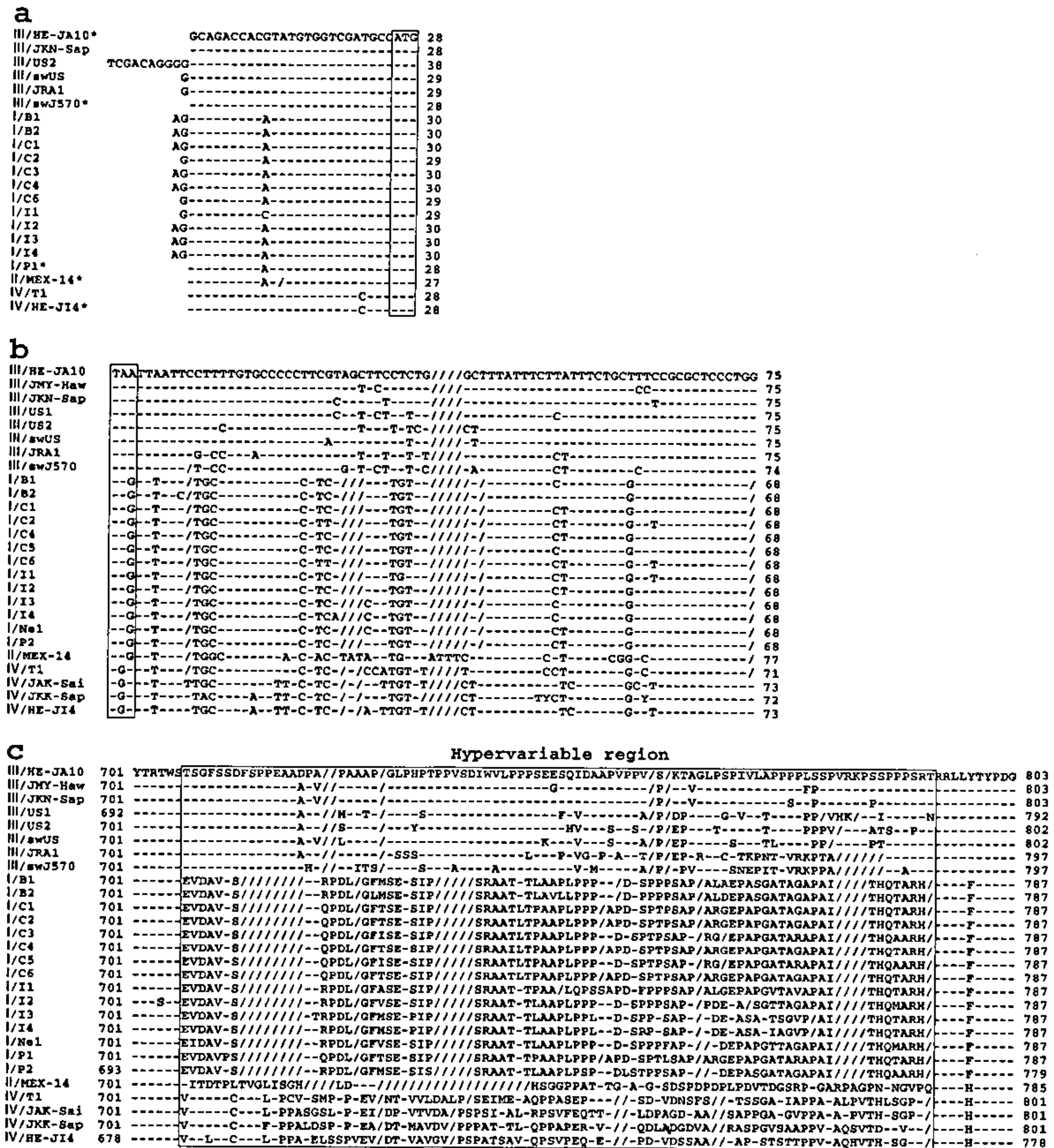

**Fig. 1.** For legend see page 424.

it is very likely that functional HEV genomes of all four genotypes have the extreme 5′ end sequence starting with GCAGACCAC and that the extra nucleotides of G, AG or TCGACAGGGG at the very extreme 5′ end are not essential. The 3′ UTR of the HE-JA10 genome consisted of 72 nt and this region showed appreciable diversity, differing by 4·2–19·4 % compared with the seven HEV isolates of genotype III and by 26·4–36·8 % compared with the 18 HEV isolates of the other three genotypes whose extreme 3′ end sequences are known (Fig. 1b). In the amino acid sequences of ORFs 1–3, the HE-JA10 isolate also had highest identities with JMY-Haw at 99·1, 99·2 and 98·4 %, respectively. As illustrated in Fig. 1(c), HE-JA10 was most closely related to JMY-Haw and JKN-Sap in the amino acid sequence of the hypervariable region of the ORF1 protein, differing by only 7 aa in each case, but differing from the remaining five isolates of the same genotype in this particular region by 18–33 aa. The hypervariable region of HE-JA10 ORF1 displayed only up to 1 aa difference among the five clones obtained, in contrast with the N terminus of the E2 protein of hepatitis C virus (Hijikata *et al.*, 1991; Weiner *et al.*, 1991) and the V3 loop of the gp120 protein of human immunodeficiency virus type 1 (Meyerhans *et al.*, 1989).

When the common 299 nt sequence of ORF2 of HE-JA10 was compared with those of the 35 human and 35 swine strains of genotypes I–IV (see Fig. 2 for names of strains), the HE-JA10 isolate shared a nucleotide identity of between 72·3 and 98·3 %. Of interest, HE-JA10 shared nucleotide identities of between 91·6 and 95·7 % with 27 genotype III HEV isolates obtained from pigs in different geographical regions of the United States (Huang *et al.*, 2002). The phylogenetic tree constructed based on the partial ORF2 sequence of 299 nt confirmed that HE-JA10 belonged to genotype III and that it was segregated into a cluster consisting of four human HEV isolates of Japanese origin (JKN-Sap, JMY-Haw, HE-JA4 and HE-JA8), with a nucleotide identity of between 96·7 and 98·3 % (Fig. 2). Another cluster consisting of five human (JRA1, HE-JA5, HE-JA6, HE-JA9 and HE-JA11) and two swine (swJ570 and swJ681) HEV isolates of Japanese origin was recognized in the other branch of genotype III.

In the present study, it was found that a genotype III HEV strain was present in Japan in the early 1990s before the emergence of a novel HEV variant of genotype III (US1) in a 62-year-old white male who contracted acute hepatitis in 1995 in the United States (Kwo *et al.*, 1997; Schlauder *et al.*, 1998); this was the first case to be identified in an industrialized country from a patient who had no history of travel to endemic areas and who was infected with a non-Asian/African HEV strain of genotype III or IV. The infected patient in the current study also had no history of travel to areas endemic for HEV and did not report contact with persons who had travelled to endemic areas or contact with farm pigs or rodents, although there are accumulating lines of evidence that animals may act as natural hosts of HEV (Erker *et al.*, 1999; Hsieh *et al.*, 1999; Huang *et al.*, 2002; Meng *et al.*, 1997, 1998, 2002; Pina *et al.*, 2000; Wang *et al.*, 2002; Wu *et al.*, 2002). The increasing globalization of food markets and increased overseas travel for business and pleasure have increased the potential of introducing HEV from not only developing countries but also industrialized countries into Japan. Japanese people have a habit of eating raw fish and other uncooked seafood, both those caught in Japan and those imported from many countries in the world, including the United States, Europe, Argentina, New Zealand and Taiwan, where HEV of genotype III is known to circulate (Garkavenko *et al.*, 2001; Pina *et al.*, 2000; Schlauder *et al.*, 1999, 2000; Wang *et al.*, 2001; Worm *et al.*, 2000). The HE-JA10 isolate obtained from our patient was nearer to the human and swine strains isolated in the United States (US1, US2, swUS and swUS01–swUS27), with the highest identity of 95·7 % in the 299 nt sequence of ORF2, than to human or swine isolates from Taiwan and Europe, indicating that HE-JA10 may be an American-like strain. However, HE-JA10 shared a nucleotide identity of 98·3 % with HE-JA8, which was recovered in 2001 from a Japanese patient who lived in Iwate and who had never been abroad: Iwate is located in the northern part of Honshu Island and is

**Fig. 1.** (a) Comparison of the 5′-terminal sequences of HEV isolates. The 5′-terminal sequences of 21 HEV isolates whose extreme 5′ end sequences are available are aligned for comparison. The initiation codon of ORF1 is boxed. In addition to the HE-JA10 isolate obtained in the present study, four isolates whose extreme 5′ end sequence had also been determined by the RLM-RACE technique are indicated by asterisks. (b) Comparison of the 3′-terminal sequences of HEV isolates. The 3′-terminal sequences of 26 HEV isolates whose sequences preceding the poly(A) tract at the 3′ terminus are available are aligned for comparison. The termination codon of ORF2 is boxed. (c) Comparison of the amino acid sequences of the hypervariable region in the ORF1 protein of HEV isolates. The sequences of the hypervariable region of the ORF1 protein of 28 HEV isolates whose entire or nearly entire sequences are available are aligned for comparison. Genotype numbers I–IV are in accordance with the recent report by Schlauder & Mushahwar (2001) and are indicated before the slash of each isolate name. Dashes indicate nucleotides/amino acids that are identical to the top sequence, while slashes denote deletions of nucleotides/amino acids. The accession nos of the 28 isolates are as follows: AB089824 (HE-JA10 isolate); AB074920 (JMY-Haw); AB074918 (JKN-Sap); AF060668 (US1); AF060669 (US2); AF082843 (swUS); AP003430 (JRA1); AB073912 (swJ570); AF051830 (Ne1); AF076239 (I3); AF185822 (P2); AF459438 (I4); D10330 (B2); D11092 (C1); D11093 (C4); L08816 (C5); L25547 (C2); L25595 (C6); M73218 (B1); M80581 (P1); M94177 (C3); X98292 (I1); X99441 (I2); M74506 (MEX-14); AJ272108 (T1); AB074915 (JAK-Sai); AB074917 (JKK-Sap); and AB080575 (HE-JI4).

approximately 450 km from Tokyo where the 'HE-JA10' patient lived. In addition, HE-JA10 shared between 96·1 and 98·3 % identity in the 412 nt sequence of ORF2 with the four recently reported Japanese HEV isolates of genotype III (HE-JA4, HE-JA8, JMY-Haw and JKN-Sap) (Takahashi *et al.*, 2002a; Mizuo *et al.*, 2002), suggesting that it is likely that genotype III HEV isolates had already been domestic in the early 1990s and were widespread in the past in Japan;

**Fig. 2.** Phylogenetic tree constructed using the neighbour-joining method based on the partial nucleotide sequence (299 nt; nt 6039–6337 of the HE-JA10 genome) of the ORF2 region of 71 human and swine HEV isolates. In addition to the 27 reported human and swine HEV isolates of genotypes I–IV whose entire or nearly entire sequences are known, 43 reported isolates of genotype III (accession nos are indicated in parentheses) whose partial sequences of 300, 301, 304, 412, 421 or 436 nt have been determined, as well as the HE-JA10 isolate obtained in the present study (boxed), were included for comparison. For simplicity, the abbreviated names (swUS01–swUS27) are used for the 27 swine strains (isolated in the United States) reported by Huang *et al.* (2002). All human and swine HEV strains isolated in Japan are indicated by bold typeface for visual clarity. Bootstrap values are indicated for the major nodes as a percentage of the data obtained from 1000 resamplings (Felsenstein, 1985).