TABLE 2
CHARACTERISTICS OF THE FIVE PATIENTS WITH ACUTE HEPATITIS C

Patient	Bed No.	Age (y)*	Gender	Cause of End-Stage Renal Failure	Duration of Hemodialysis	Genotype	HCV RNA Level	
							At Diagnosis	At Start of IFN†
A	16	50	Male	CGN	26 y, 9 mo	1b	< 0.2	< 0.2
B	17	53	Male	PCK	4 y, 2 mo	1b	< 0.2	< 0.2
C	18	52	Male	CGN	6 y, 9 mo	1b	< 0.2	< 0.2
D	19	60	Male	CGN	14 y, 3 mo	1b	4.2	6.0
E	20	67	Female	CGN	2 y, 8 mo	1b	< 0.2	< 0.2

HCV = hepatitis C virus; IFN = interferon; CGN = chronic glomerulonephritis; PCK = polycystic kidneys.
*At diagnosis of acute hepatitis C.
†First IFN treatment for patient E.

sequence analysis was performed on the same 2 days for those 9 patients. The 5'NC (120 bp, nucleotides 86 to 206, numbered as reported by Okamoto et al.[18]) and core (57 to 126 aa) regions of HCV genotype 1b were amplified as described elsewhere.[19] Products of the second PCR were purified from agarose gel and cloned with pT7 Blue T-Vectors (Novagen, Inc., Madison, WI). Five independent clones of the two target sizes were isolated from each patient's sample and were sequenced using the dideoxynucleotide method with an ABI automated sequencer (PE-Applied Biosystems, Foster City, CA).

RESULTS

Outbreak of Acute Hepatitis C

In the 4 months before this outbreak, 35 patients had received dialysis on shifts 1 and 2 in the same room as the 5 patients with acute hepatitis C (patients A, B, C, D, and E). Of the 35 patients, 12 were chronically infected with HCV. Of the 12 patients, 9 were infected with HCV genotype 1b, 1 with genotype 2a, and 2 with an undeterminable genotype (Table 1). The 5 patients were diagnosed as having acute hepatitis C and were found to have HCV viremia with elevation of alanine aminotransferase level (mean, 224.4 IU/L; range, 100 to 422 IU/L) and be negative for anti-HCV on September 11, 2000 (Fig. 1). Table 2 lists patient characteristics. In May 2000, all 5 had had a regular screening test with anti-HCV and were HCV RNA negative. These 5 patients were infected with HCV genotype 1b, and all were negative for IgM hepatitis A antibody, hepatitis B surface antigen, IgM hepatitis B core antibody, hepatitis G virus RNA, and TT virus DNA. None of the patients had jaundice or other symptoms. Anti-HCV became positive 3 to 5 months later than HCV RNA in the 5 patients. None of the patients had a history of prior medical intervention such as general or orthopedic surgery, blood transfusion, or hepatitis infection or other risk factors for the acquisition of HCV including surgery, tattooing or body piercing, dental procedures, intravenous-drug use, and high-risk sexual behavior. None of the patients had a family history of hepatitis. The seroconversion and clinical data suggested that the 5 patients were infected on the same occasion and that there was possibly a single source of transmission.

TABLE 3
VARIATIONS IN THE 5' NONCODING REGION AT NUCLEOTIDE POSITIONS 90, 102, AND 187 IN THE 5 PATIENTS WITH ACUTE HEPATITIS C AND 9 OTHER PATIENTS WITH CHRONIC HEPATITIS C VIRUS GENOTYPE 1B VIREMIA

Shift	Bed No.*	State of HCV Infection	Nucleotide Position†		
			90	102	187
1	2	Chronic	G	T	C
	3	Chronic	G	A	T
	6	Chronic	G	A	C
	7	Chronic	A	C	A
	17	Chronic	G	A	C
	18	Chronic	G	A	C
	20	Chronic	A	A	C
2	4	Chronic	G	T	C
	15	Chronic	A	T	T
	16, patient A	Acute	A	A	C
	17, patient B	Acute	A	A	C
	18, patient C	Acute	A	A	C
	19, patient D	Acute	A	A	C
	20, patient E	Acute	A	A	C

HCV = hepatitis C virus.
*Bed locations are shown in Figure 2.
†Nucleotides were numbered from the presumptive 5'-noncoding region of the HC-J2 strain.[18]

Sequence Analysis of 5'NC and Core Regions

To determine the source of transmission of HCV, we performed serial sequence analysis of the 5'NC and core regions of HCV from sera 1 and 2 weeks after elevation of alanine aminotransferase level in the 5 patients with acute hepatitis C, and compared the results with those of the same analysis in 9 patients with chronic HCV genotype 1b viremia.

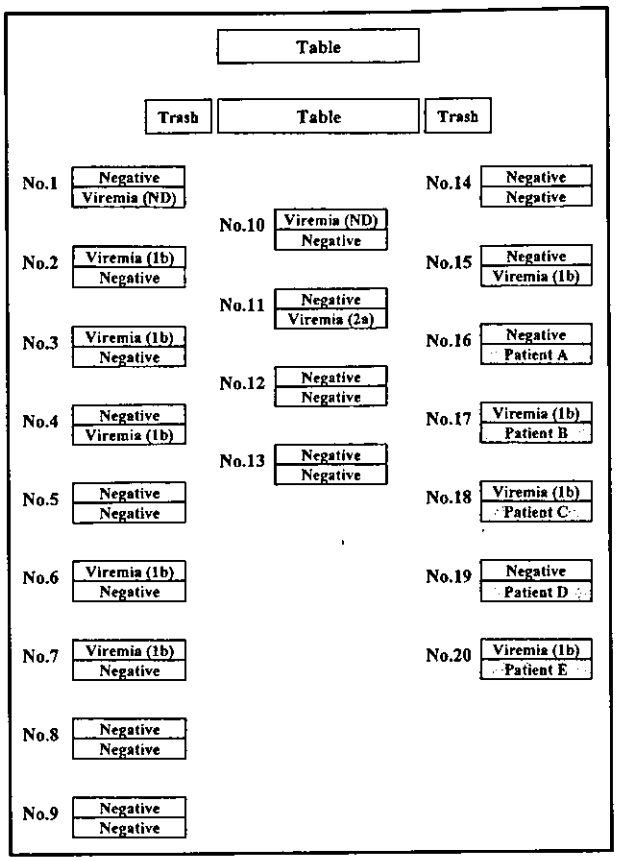

FIGURE 2. Location of the 20 beds in the hemodialysis unit. These were occupied by the 5 patients with acute hepatitis C and the other 35 patients, including those with chronic hepatitis C virus (HCV) viremia, during shifts 1 and 2. Shifts 1 and 2 are indicated by the upper and lower rectangles of each bed, respectively. Viremia means patients with positivity for both antibody to HCV and HCV RNA, and the HCV genotype is shown in parentheses. Negative means patients who were negative for HCV RNA, despite positivity or negativity for antibody to HCV. HCV markers are listed in Table 1. ND = not determinable.

In the sequence analysis of the 5'NC region, consistency was found in the 5 independent clones from each patient with acute hepatitis C and those from each patient with chronic HCV viremia. None of the patients had a heterogeneous viral population (quasispecies). Moreover, no quasispecies were found over time in the clones of any of 14 analyzed patients. In all, 6 variations in the 5'NC region were identified with point mutation at nucleotide positions 90, 102, and 187 (Table 3). The 5 patients with acute hepatitis C had the same sequencing in the 5'NC region. The only other patient who shared the same sequence was in bed 20 during shift 1. It was concluded that this patient with chronic HCV viremia must have been the source of the outbreak.

In contrast, the sequence analysis of the core region of 5 independent clones from each patient showed independent genetic clusters (quasispecies) in each patient and further quasispecies over time, between September 18 and 25, 2000. We were not able to determine the source virus by the core region sequence.

Hemodialysis Situation

Figure 2 shows the location of the 20 hemodialysis beds that were used by the patients. All 5 patients with acute hepatitis C underwent hemodialysis on shift 2 and used adjacent beds 16, 17, 18, 19, and 20. The one who was in bed 20 on shift 2 shared the same bed as the probable source-patient. In the hemodialysis room, trash receptacles for medical waste existed only near the tables for preparing hemodialysis.

A thorough investigation of the patient charts showed that among all 5 patients with acute hepatitis C whose visits to the unit overlapped was the source-patient; the source-patient (shift 1, bed 20) often underwent hemodialysis before the 5 patients with acute hepatitis C, and most of their hemodialysis-related medicine was prepared during the source-patient's treatment for the period from May to September 11, 2000.

Each patient received hemodialysis through an indwelling catheter that was slowly flushed with heparin–saline during treatment. In this unit, a single, small (20-mL) saline ampoule was used for heparin solution to avoid the possibility that a large bottle might accidentally be shared among patients. However, the ampoule had a recap function and it was difficult to determine whether the ampoule was new or had already been used. Concerning the patient in bed 20 during shift 1, it was difficult for staff members to start indwelling catheters for hemodialysis for him because of the form of artery–vein shunt in his arm; this led to more time and heparin–saline solution than that required for other patients.

Given the above, it was considered likely that the 5 patients with acute hepatitis C may have shared a single heparin–saline solution ampoule contaminated by HCV from the source-patient while undergoing hemodialysis.

Clinical Course of the Patients With Acute Hepatitis C

HCV viremia continued to be detectable until 51, 166, 159, 51, and 51 days after the outbreak in patients A, B, C, D, and E, respectively, all of whom nonetheless went on to achieve spontaneous viral clearance. Alanine aminotransferase levels tended to decrease and nearly normalized, but remained above normal limits (less than 35 IU/L). All of the patients first received natural interferon-beta monotherapy, 3 megaunits thrice weekly for 6 months. The therapy was effective from a virologic and biochemical standpoint for 4 patients (patients A, B, C, and D), whose alanine aminotransferase levels normalized and results on HCV RNA tests quickly became negative after the start of the scheduled therapy; the negativity was sustained for 6 months after treatment. Patient E was resistant to interferon-alpha after treatment with interferon-beta and was not able to clear HCV viremia (Fig. 1).

DISCUSSION

Hemodialysis patients are involved in ongoing chains of HCV infection by apparently unknown nosoco-

mial modes.[9] Little data are available to document the mode of transmission. The current study showed the value of molecular techniques in providing evidence of the occurrence of an HCV outbreak and for documenting the transmission of HCV infection in a hemodialysis unit. To our knowledge, this is the first demonstration that a high degree of homology in the sequences of the HCV 5'NC region could be used to trace the source of transmission of this type of infection.

Acute hepatitis C often remains undiagnosed during the acute phase, which is usually asymptomatic.[20] Therefore, the prospective observation of cohorts of individuals in whom acute hepatitis C has been recognized at an early stage would supply valuable information on the short-term outcome of the infection. We were able to document an outbreak of nosocomial HCV infection in a hemodialysis unit because the patients had been studied prospectively since 1989 to investigate the epidemiology of HCV infection.[9-13]

The current study showed the usefulness of molecular techniques for the investigation of HCV outbreaks. Molecular evidence of HCV infection has been reported in several studies based on sequencing of the functional regions of the HCV genome (hypervariable region 1 and the NS5B region, from which the encoded proteins are thought to come under immune pressure[21-23]) and the other regions.[24-26] The 5'NC region of the HCV genome, which has an essential function in the initiation of viral protein translation, is highly conserved between virus isolates in both the primary sequence and the predicted secondary structure, but there is some variation in the region even between different isolates of the same HCV genotype.[27] Sequence homology of the 5'NC region of HCV indicated an indigenous strain in all 5 newly infected patients and transmission from a single patient with chronic HCV viremia. HCV is genetically heterogeneous and is characterized by the existence of a quasispecies distribution of the viral population within a single infected individual, as the viral RNA polymerase is devoid of any proofreading capacity.[28] Our previous studies showed that patients with chronic HCV viremia and consistently abnormal levels of alanine aminotransferase had quasispecies of the HCV core region that changed over time, but patients with consistently normal levels of alanine aminotransferase had identical and highly conserved core regions.[29,30] The patients in the current outbreak all had high elevation of alanine aminotransferase levels and had intra-patient and time-related quasispecies, identified by the core region sequencing. On the other hand, sequence report within the HCV E1 and E2 envelope regions indicated the evolutionary dynamics of the HCV quasispecies during the acute phase of HCV.[31] Genomic sequencing of the 5'NC region of the HCV was useful for finding the putative source of the outbreak, but genomic sequencing of the core region was not.

Nosocomial HCV infection in hemodialysis units has been reported to be due to poor sterilization of dialysis machines.[32] In our previous study measuring HCV RNA from inlet and outlet samples of the dialyzers of patients positive for HCV RNA, the detection in the filtrate of patients indicated that backflow in the inlet and outlet on startup and shutdown of the machine might be a possible route of nosocomial infection.[9] However, the possibility of HCV passage across the dialysis membrane remains controversial.[33] Patients commonly receive hemodialysis through indwelling catheters that are flushed with heparin–saline during treatment. With strict adherence to standard precautions delineated by the Centers for Disease Control and Prevention,[34] medicines were prepared individually for each patient in the unit studied. Among all 5 patients with acute hepatitis C whose visits to the unit overlapped was the source-patient. HCV transmission likely occurred when treatments for several patients were performed at the same time. In the hemodialysis unit, trash receptacles for medical waste existed only near the tables for preparation. This may have led to the staff mistakenly reusing the heparin solution ampoule with the recapping function. The epidemiologic interviews and molecular analysis in the current study indicated the possibility that the 5 patients with acute hepatitis C had shared a single heparin–saline solution ampoule contaminated by the HCV of the source-patient. Improvements in infection control procedures, such as placing a trash receptacle near each bed and prohibiting reuse of the ampoule, are necessary.

The patients with acute hepatitis C started interferon therapy within 6 months after infection. All but one had a sustained response to interferon because of their low level of HCV RNA and the relatively early start of therapy. In a prospective study of chronic HCV viremia, we demonstrated lower serum levels of HCV RNA in hemodialysis patients than in non-uremic patients, probably because maintenance hemodialysis decreased the levels of HCV, but never to the point of total clearance.[10] This finding suggested high rates of sustained HCV elimination in hemodialysis patients treated with interferon. Izopet et al.[35] reported elimination rates of 42% to 64% in a study of interferon therapy for hemodialysis patients. Moreover, early treatment of acute hepatitis C with interferon has an extremely high probability of preventing chronic infection in non-uremic patients.[36] Although the number of patients with acute hepatitis C treated with interferon in our study was small, interferon appeared to produce a host–virus interaction within months of the primary HCV infection, probably leading to the desired outcome of HCV elimination in these hemodialysis patients.

Sequence analysis of the HCV 5'NC region was useful for determining the source of another outbreak of HCV infection in our hemodialysis unit. Nosocomial HCV infection can result from poor infection control practice when treatment is performed for several patients at the same time.

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Carotid atherosclerosis and cardiovascular risk factors: a comparison of residents of a rural area of Okinawa with residents of a typical suburban area of Fukuoka, Japan

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Abstract

Areas of Japan are known worldwide for the longevity of their residents. Okinawa has the highest longevity in Japan and a low rate of death due to cardiovascular disease. We investigated carotid atherosclerotic (CA) risk factors in islands of I city in Okinawa prefecture and compared them with K town, a suburban area of Fukuoka prefecture in Kyushu, to determine the relationship between cardiovascular risk factors and carotid atherosclerosis. We investigated conventional cardiovascular risk factors in 1078 I city residents (375 men, mean age 63.7 and 703 women, mean age 60.0) in 2000 and 2364 K town residents (676 men, mean age 57.1 and 1688 women, mean age 53.0) in 1999. Carotid atherosclerosis was assessed by mean intima-media thickness (IMT) by B-mode ultrasound. The mean IMT was significantly lower in the residents of I city than in those of K town ($P < 0.05$). Total cholesterol (TC) and low-density-lipoprotein cholesterol (LDL-C) levels and smoking rate were also lower in I city than in K town. Body mass index (BMI) and triglyceride (TG) level were higher in I city than in K town. In I city, multiple regression analysis found independent relationships between carotid atherosclerosis and age, sex (male), hypertension, LDL cholesterol, high-density-lipoprotein cholesterol (HDL-C), and diabetes. The lower mean IMT is probably related to a lower lifetime burden of atherosclerotic risk factors, which may in turn be related to the longevity of Okinawa residents. BMI was not a cardiovascular risk factor, although LDL cholesterol was a common important risk factor.

Keywords: Okinawa; Fukuoka; Japan; Carotid arteries; Risk factors; Cross-sectional study

1. Introduction

Japan is known worldwide for the longevity of its residents. Okinawa is known for having the highest longevity in Japan and a low rate of death due to cardiovascular disease, including coronary heart disease and stroke, which are leading causes of death in many countries [1–3]. We thought, therefore, that an investigation of Okinawa residents would be useful for identifying factors that could be useful in the prevention of cardiovascular disease.

B-mode ultrasonography currently appears to provide the most accurate assessment of early atherosclerosis, allowing visualization and direct measurement of wall thickness. An increase in carotid artery intima-media thickness (IMT) has been associated with conventional cardiovascular risk factors [4–6], coronary heart disease, stroke, and atherosclerosis elsewhere in the arterial system [7–9]. IMT abnormalities are also predictive of cardiovascular prognosis [10,11] and improvement of carotid atherosclerosis (CA) has been reported to reduce cardiovascular events [12]. On the basis of these findings, carotid IMT measurement can be regarded as an indicator of generalized atherosclerosis. Moreover, noninvasive assessment of IMT makes ultrasonography ideal for screening and for serial studies [13].

Little study has been done of the relationship between cardiovascular risk factors and carotid atherosclerosis in Okinawa. We investigated carotid atherosclerotic risk factors in the general population of I city, located on an island in the eastern part of the Yaeyama district of Okinawa prefecture [14,15] and compared the results with K town, a typical

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Japanese suburban city in Fukuoka prefecture [16], to determine the relationship between cardiovascular risk factors and carotid atherosclerosis.

2. Materials and methods

2.1. Subjects

The studies were approved by the Ethics Committee of Kyushu University Hospital. Informed consent was obtained from all residents.

We investigated 1078 I city residents in 2000 and 2353 K town residents in 1999 without a history of cardiovascular disease, including coronary heart disease and stroke, who participated in free health examinations. Of the 1078 I city residents analyzed, 375 were men and 703 were women, aged 20–89. Of the 2364 K town residents analyzed, 676 were men and 1688 were women, aged 20–89. After exclusion for hyperlipidemia, hypertension, diabetes and obesity, 62 men and 205 women in I city and 184 men and 699 women in K town were categorized as healthy subjects.

2.2. Medical history

Excluded were subjects who had a history of coronary heart disease and/or stroke, refused ultrasound examination, did not return the questionnaire, or did not take the required blood examination. Altogether, 50 men and 81 women from among the 1209 subjects of I city and 46 men and 62 women from among the 2472 subjects from K town were excluded from this analysis. Height and weight were measured in light clothing and without shoes. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Systolic blood pressure and diastolic blood pressure were twice measured in the right arm with the subject in a sitting position after taking a short rest. Smoking behavior was assessed by questionnaire. Subjects were classified as smokers (current or past smokers) and non-smokers. Hypertension was defined as either systolic blood pressure $\geq$140 mmHg, diastolic pressure $\geq$90 mmHg, or treatment with antihypertensive medications. Hyperlipidemia was defined as either total cholesterol (TC) $\geq$220 mg/dl, triglyceride (TG) $\geq$150 mg/dl, or lipid-lowering drug administration. Diabetes was defined as a self-reported history of diabetes, a fasting plasma glucose (FPG) level $\geq$126 mg/dl, or the use of antidiabetic drugs. Obesity was defined as BMI $\geq$26.4 kg/m^2.

2.3. Assay methods

All blood samples were drawn between 9 and 12 a.m. after an overnight fast and stored at −20 °C until analysis. The following parameters were measured. Total cholesterol, high-density-lipoprotein cholesterol (HDL-C), triglyceride and fasting plasma glucose. Low-density-lipoprotein cholesterol (LDL-C) was calculated indirectly using the Friedewald formula.

2.4. Ultrasonographic measurement

High-resolution B-mode ultrasound examination was done with a 7.5 MHz mechanical sector transducer on the Aloka SSD-2000 (Aloka Co. Ltd., Tokyo, Japan) by three specially trained ultrasound technicians. All the medical histories and the assay results were blinded to the ultrasound technicians. Carotid IMT was measured at points 20, 25 and 30 mm proximal to the flow divider on the far wall of the right and left common carotid artery at the end of the diastolic phase. From this, mean IMT was determined for each individual. Mean IMT $\geq$1.1 mm was defined as abnormal IMT. Carotid atherosclerosis was defined as the presence of abnormal IMT.

2.5. Statistical analysis

Age, sex, BMI, total cholesterol level, LDL cholesterol level, HDL cholesterol level, triglyceride level, systolic blood pressure, diastolic blood pressure, diabetes and smoking rate were used as conventional risk factors and mean IMT was used to define the level of carotid atherosclerosis. Mean numerical variable comparison between I city and K town by age groups categorized 20–39, 40–49, 50–59, 60–69 and 70–89 was done by Student's t-test. Categorical variable comparison between I city and K town by age was done by the χ^2-test. Two way ANOVA was used for the means of numerical variables and Mantel Haenszel test was used to adjust categorical variables for age in comparison of I city and K town. Forward stepwise multiple logistic regression analysis was used to determine the independent risk factors for carotid atherosclerosis. P values <0.05 were considered statistically significant.

3. Results

Area profiles by age and sex are shown in Tables 1 and 2. Both male and female mean IMT was significantly lower in I city than in K town ($P < 0.05$, 0.05, respectively, two way ANOVA).

In comparison of I city and K town, the conventional risk factors total cholesterol, HDL cholesterol and LDL cholesterol were significantly lower in both male and female I city residents ($P < 0.05$, 0.05, 0.05 in males and $P < 0.05$, 0.05, 0.05 in females, respectively, two way ANOVA). Triglyceride level and BMI were significantly higher in both male and female I city residents ($P < 0.05$, 0.05 in males and $P < 0.05$, 0.05 in females, respectively, two way ANOVA). The difference in BMI between I city and K town increased as age increased. Systolic blood pressure was significantly lower in I city females ($P < 0.05$; two way ANOVA). The diabetes rate was significantly higher in male I city

Table 1
Characteristics of the male study population by age and area

	Age group					Total
	20–39	40–49	50–59	60–69	70–89	
Number						
I city	22	49	36	120	148	375
K town	91	110	116	229	130	676
BMI (kg/m^2)						
I city	23.6 (3.9)	25.0 (3.4)[a]	24.4 (3.2)	24.4 (2.6)[a]	23.5 (3.1)[a]	24.1 (3.1)[b]
K town	23.3 (3.8)	23.1 (2.8)	23.8 (2.6)	23.5 (2.9)	22.4 (2.9)	23.2 (3.0)
SBP (mmHg)						
I city	118.8 (13.6)	126.9 (15.0)[a]	127.3 (16.7)	132.4 (14.6)[a]	132.6 (17.2)[a]	130.4 (16.2)
K town	115.4 (11.9)	119.7 (15.1)	130.4 (17.2)	136.8 (18.0)	139.0 (18.8)	130.5 (19.0)
DBP (mmHg)						
I city	77.4 (10.5)[a]	83.2 (11.7)[a]	82.9 (12.2)	79.3 (8.8)	73.5 (10.7)[a]	77.8 (11.0)
K town	71.8 (9.7)	77.5 (11.7)	81.6 (11.1)	81.3 (11.3)	77.7 (9.8)	78.7 (11.3)
TC (mg/dl)						
I city	193.5 (32.2)	197.4 (33.7)	201.4 (29.5)[a]	194.1 (30.0)[a]	192.6 (28.3)[a]	194.6 (29.9)[b]
K town	201.8 (33.6)	208.1 (37.5)	215.0 (37.1)	203.5 (33.7)	204.0 (32.2)	206.1 (34.8)
HDL-C (mg/dl)						
I city	54.6 (12.5)	53.6 (12.7)	55.3 (10.9)	55.0 (12.9)	56.0 (13.9)	55.2 (13.0)[b]
K town	55.7 (15.0)	56.3 (13.2)	56.4 (14.6)	56.8 (14.7)	57.4 (14.1)	56.6 (14.4)
LDL-C (mg/dl)						
I city	110.7 (29.6)	110.6 (33.5)[a]	117.2 (26.8)[a]	113.1 (26.6)[a]	113.1 (26.1)[a]	113.0 (27.8)[b]
K town	121.3 (28.9)	126.3 (32.6)	129.9 (33.3)	122.2 (31.6)	124.6 (28.9)	124.5 (31.2)
TG (mg/dl)						
I city	141.4 (78.5)	165.9 (79.4)[a]	144.9 (76.3)	130.2 (70.8)	117.8 (66.5)	132.0 (72.7)[b]
K town	124.2 (68.3)	127.5 (68.7)	143.5 (90.4)	122.2 (65.8)	110.2 (55.9)	124.7 (70.3)
Diabetes rate (%)						
I city	4.5[c]	12.2	13.9	19.2[c]	12.2	14.1[d]
K town	0.0	1.8	10.3	10.9	14.6	8.6
Smoking rate (%)						
I city	54.5[c]	67.3[c]	36.1[c]	45.0[c]	49.3[c]	49.3
K town	78.0	87.3	75.9	74.7	89.2	80.2
Current						
I city	45.5	44.9	27.8	32.5	29.7	33.3
K town	63.7	49.1	39.7	28.8	30.8	39.1
Past						
I city	9.1	22.4	8.3[c]	12.5[c]	19.6[c]	16[d]
K town	14.3	38.2	36.2	45.9	58.5	41.1
Mean IMT (mm)						
I city	0.57 (0.14)[a]	0.68 (0.16)	0.74 (0.16)[a]	0.83 (0.18)[a]	0.93 (0.24)[a]	0.83 (0.22)[b]
K town	0.66 (0.13)	0.72 (0.13)	0.83 (0.19)	0.94 (0.20)	1.03 (0.26)	0.86 (0.23)

Values in parentheses are mean (S.D.). BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, high-density-lipoprotein cholesterol; LDL-C, low-density-lipoprotein cholesterol; TG, triglyceride.

[a] $P < 0.05$ between I city and K town in the same age group by Student's t-test.

[b] $P < 0.05$ between I city and K town adjusted for age by two way ANOVA.

[c] $P < 0.05$ between I city and K town in the same age group by χ^2-test.

[d] $P < 0.05$ between I city and K town adjusted for age by Mantel Henszel test.

residents ($P < 0.05$; Mantel Haenszel test) and the smoking rate was significantly lower in both male and female I city residents ($P < 0.05$, 0.05, respectively, Mantel Haenszel test).

Forward stepwise multiple logistic regression analysis (Table 3) showed age, high LDL cholesterol level, low HDL cholesterol level, sex (male), and diabetes to be independent risk factors for carotid atherosclerosis (mean IMT $\geq$ 1.1 mm) in I city ($P < 0.0001$, OR; 2.13, $P = 0.0005$, OR; 1.02, $P = 0.0012$, OR; 0.96, $P = 0.0023$, OR; 2.34, $P = 0.0064$, OR; 2.49, respectively). Age, high systolic blood pressure, high LDL cholesterol level, sex (male),

Table 2
Characteristics of the female study population by age and area

	Age group					Total
	20–39	40–49	50–59	60–69	70–89	
Number						
I city	73	111	110	199	210	703
K town	312	344	429	423	180	1688
BMI (kg/m^2)						
I city	21.5 (3.4)	23.5 (3.7)[a]	24.6 (3.5)[a]	24.7 (2.8)[a]	24.5 (3.5)[a]	24.1 (3.5)[b]
K town	20.9 (3.2)	22.1 (3.1)	22.4 (2.9)	23.2 (3.2)	22.2 (3.4)	22.2 (3.2)
SBP (mmHg)						
I city	107.2 (14.0)	116.3 (16.1)	123.9 (14.7)	127.5 (16.1)[a]	133.4 (15.9)[a]	124.8 (17.6)[b]
K town	108.1 (12.6)	115.9 (15.3)	127.9 (20.2)	134.3 (19.4)	136.7 (16.4)	124.4 (20.3)
DBP (mmHg)						
I city	66.3 (10.4)	73.0 (12.1)	76.2 (10.5)	75.5 (10.3)[a]	74.6 (10.0)	74.0 (10.9)
K town	66.6 (9.1)	71.2 (10.3)	77.6 (11.3)	78.6 (10.3)	75.8 (8.5)	74.3 (11.2)
TC (mg/dl)						
I city	174.4 (26.5)[a]	195.0 (32.8)	214.4 (31.9)[a]	216.2 (35.4)	214.3 (33.4)[a]	207.7 (35.6)[b]
K town	182.4 (29.1)	202.0 (32.7)	227.9 (38.2)	228.9 (35.8)	219.4 (29.9)	213.5 (38.5)
HDL-C (mg/dl)						
I city	64.0 (15.7)	60.6 (13.6)[a]	62.0 (13.9)[a]	55.0 (11.0)[a]	57.4 (13.2)[a]	58.6 (13.4)[b]
K town	64.6 (13.8)	65.5 (13.9)	66.1 (14.5)	62.2 (14.8)	52.7 (13.7)	64.4 (14.3)
LDL-C (mg/dl)						
I city	93.6 (25.4)[a]	113.9 (28.8)[a]	130.1 (28.1)[a]	133.5 (32.6)[a]	129.7 (30.9)	124.6 (32.5)[b]
K town	104.0 (25.8)	120.7 (30.7)	141.7 (34.1)	143.9 (33.1)	134.4 (28.2)	130.2 (34.6)
TG (mg/dl)						
I city	83.9 (56.2)[a]	102.6 (51.2)[a]	111.6 (62.8)	138.7 (66.1)[a]	135.9 (71.1)[a]	122.2 (66.7)[b]
K town	69.0 (37.6)	78.6 (39.6)	100.6 (57.8)	113.8 (57.7)	111.5 (60.8)	94.8 (54.4)
Diabetes rate (%)						
I city	0.0	3.6	3.6	7.5	10.5	6.4
K town	0.6	1.5	4.2	5.4	6.7	3.6
Smoking rate (%)						
I city	16.4	7.2[c]	5.5	2.5[c]	3.3[c]	5.4[d]
K town	26.6	21.5	10.3	8.3	9.4	15.0
Current						
I city	12.3	5.4	5.5	2.0	1.9	4.1[d]
K town	15.1	10.8	6.5	5.0	3.9	8.3
Past						
I city	4.1	1.8[c]	0.0[c]	0.5[c]	1.4[c]	1.3[d]
K town	11.5	10.8	3.7	3.3	5.6	6.7
Mean IMT (mm)						
I city	0.54 (0.10)[a]	0.64 (0.12)[a]	0.72 (0.13)[a]	0.80 (0.16)[a]	0.87 (0.19)[a]	0.75 (0.19)[b]
K town	0.61 (0.11)	0.69 (0.12)	0.78 (0.15)	0.89 (0.17)	0.95 (0.18)	0.77 (0.19)

Values in parentheses are mean (S.D.). BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, high-density-lipoprotein cholesterol; LDL-C, low-density-lipoprotein cholesterol; TG, triglyceride.
[a] $P < 0.05$ between I city and K town in the same age group by Student's t-test.
[b] $P < 0.05$ between I city and K town adjusted for age by two way ANOVA.
[c] $P < 0.05$ between I city and K town in the same age group by χ^2-test.
[d] $P < 0.05$ between I city and K town adjusted for age by Mantel Henszel test.

high triglyceride level, and high diastolic blood pressure were independent risk factors for carotid atherosclerosis (mean IMT $\geq$ 1.1 mm) in K town ($P < 0.0001$, OR; 2.56, $P < 0.0001$, OR; 1.03, $P < 0.0001$, OR; 1.01, $P < 0.0001$, OR; 2.09, $P = 0.0115$, OR; 1.00, $P = 0.0478$, OR; 0.98, respectively).

On analysis of healthy subjects (Table 4), the mean IMT of female I city residents was significantly lower than that found in K town residents ($P < 0.05$; two way ANOVA). The mean IMT of male I city residents was lower in each age group than was found in K town males, but there was no significant difference by two way ANOVA.

Table 3
Forward stepwise multiple logistic regression analysis of CA (mean IMT 1.1 mm) by age, sex, BMI, TC, HDL-C, LDL-C, TG, SBP, DBP, and diabetes rate

Variable	Coefficient	Odds ratio	95% CI of OR		P value
I city					
Age (year)	0.7559	2.13	1.63	2.77	0.0001
LDL-C (mg/dl)	0.0153	1.02	1.01	1.02	0.0005
HDL-C (mg/dl)	−0.0368	0.96	0.94	0.99	0.0012
Male (yes/no)	0.8487	2.34	1.35	4.05	0.0023
Diabetes (yes/no)	0.9500	2.59	1.35	4.94	0.0064
K town					
Age (year)	0.9413	2.56	2.12	3.1	<0.0001
SBP (mmHg)	0.0252	1.03	1.01	1.04	<0.0001
LDL-C (mg/dl)	0.0110	1.01	1.01	1.02	<0.0001
Male (yes/no)	0.7348	2.09	1.48	2.93	<0.0001
TG (mg/dl)	0.0032	1.00	1.00	1.01	0.0115
DBP (mmHg)	−0.0213	0.98	0.96	1.00	0.0478

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, high-density-lipoprotein cholesterol; LDL-C, low-density-lipoprotein cholesterol; TG, triglyceride; CA, carotid atherosclerosis (mean IMT 1.1 mm).

4. Discussion

The present cross-sectional study is the first to use ultrasonography to survey carotid atherosclerosis in a sub population of Okinawa residents. We showed that the intima-media thickness of I city residents was significantly lower than that of K town residents. Previous research reported that residents of Okinawa had a low rate of death due to cardiovascular disease [1–3]. According to the Japanese Ministry of Health, Labour and Welfare, mortality by coronary heart disease and stroke in Okinawa prefecture were lower than Fukuoka prefecture. In 1999, the mortality rates (per 100,000 population) for coronary heart disease and stroke were 45.9 and 58.7, respectively, in Okinawa prefecture and 53.3 and 95.0, respectively, in Fukuoka prefecture. The difference in IMT was probably related to the low rate of death due to cardiovascular disease in Okinawa.

The serum total cholesterol, LDL cholesterol and HDL cholesterol, and smoking rate were lower in the residents of I city than in K town residents and the BMI, triglyceride level and diabetes rate were higher. On multiple regression analysis, age, male sex and LDL cholesterol were independent risk factors for carotid atherosclerosis in both I city and K town. These results show LDL cholesterol to be one of the most important biochemical risk factors for atherosclerosis. In the present study, although, the LDL cholesterol level was lower in I city than in K town, the HDL cholesterol level was lower and the triglyceride level was higher in I city than in K town. In 1985, Hosaki et al. [17] reported that the residents of Okinawa had a higher serum HDL cholesterol level and a lower LDL cholesterol level than residents of Tokyo, and that this was related to the lower cardiovascular mortality seen in Okinawa. Also implicated was the possibility of a relationship between the simple Okinawan low-fat diet composed of rice, sweet potatoes, soybeans, vegetables, seaweed, pork and fish. Changes in dietary habits in Okinawa related to the American influence after World War II and the necessity to establish better dietary habits to improve health were reported [18]. The present study indicates that delicate changes in serum lipoproteins may be gradually occurring with changes in dietary habits in the rural areas of Okinawa. The mean IMTs of the younger age groups (20–39 groups) in Okinawa, however, were still lower than those of the corresponding age groups in Fukuoka. This may indicate that

Table 4
Characteristics of healthy subjects

	Age group					Total
	20–39	40–49	50–59	60–69	70–89	
Male						
Number						
I city	7	6	5	18	26	62
K town	48	38	27	46	25	184
Mean IMT						
I city	0.59 (0.16)	0.63 (0.11)	0.74 (0.11)	0.75 (0.22)[a]	0.91 (0.19)	
K town	0.63 (0.10)	0.70 (0.13)	0.81 (0.16)	0.92 (0.24)	1.00 (0.20)	
Female						
Number						
I city	56	59	32	36	22	205
K town	260	210	121	74	34	699
Mean IMT						
I city	0.54 (0.10)[a]	0.62 (0.10)[a]	0.69 (0.11)[a]	0.79 (0.14)	0.85 (0.17)[b]	
K town	0.60 (0.11)	0.68 (0.11)	0.75 (0.14)	0.84 (0.19)	0.93 (0.15)	

Values with parentheses are mean (S.D.). Healthy subjects are defined as subjects after exclusion of those with hyperlipidemia, hypertension, diabetes and obesity.

[a] $P < 0.05$ between Okinawa and Fukuoka in the same age group by Student's t-test.

[b] $P < 0.05$ between Okinawa and Fukuoka adjusted for age by two way ANOVA.

not only dietary habits but some other important factors influence carotid IMT. Future observation is needed to clarify the significance of dietary habits on serum lipoproteins and carotid IMT in Okinawa.

Obesity has become a public health problem because of its increasing prevalence and the associated cardiovascular risk factors, including diabetes, hyperlipidemia and hypertension [19]. BMI is widely used as an index of obesity [20–23], and much attention has been paid to BMI as a risk factor for coronary heart disease [24,25]. However, BMI was not a significant risk factor for carotid atherosclerosis in either area. Despite the higher BMI in I city than in K town, the residents of I city had a lower mean IMT, lower rate of cardiovascular disease and the highest longevity in Japan. The high BMI in I city tended to be most notable in the older age groups. High BMI may not be influential as a causal factor of cardiovascular disease. Some studies have reported that higher body weight in older persons is negatively correlated with the death rate [26–28]. That a higher BMI, as found in I city, has a beneficial effect for older persons and is involved in the low rate of cardiovascular disease is possible. In addition, BMI indicates neither the regional fat composition nor muscle or bone mass, even though previous studies have reported that abdominal fat as indicated by waist-to-hip circumference, computed tomography, or ultrasonography is more closely associated with atherosclerosis and cardiovascular risk factors than overall body fat as indicated by BMI [29–34]. Furthermore, some studies have reported the possibility of differences in the relationship between BMI and cardiovascular risk by race, ethnicity, and geographical area [35,36]. Residents of Okinawa are racially and ethnically different than mainland Japanese. Obesity as a risk factor for cardiovascular disease, therefore, needs to be evaluated more carefully in Japan. Further study of obesity will be needed to determine how the amount and prevalence of body fat is related to cardiovascular risk factors in Japan and Okinawa.

The average mean IMT in all age groups was also lower in I city than in K town among healthy subjects. This indicates the possibility that factors other than conventional risk factors were related to the lower IMT in Okinawa. Some environmental factors such as food, climate, or lifestyle may be related to this phenomenon. Genetic factors also may be important. Some studies have reported relationship between genetic factors, such as the endothelial nitric oxide synthase locus, LDL receptor locus, and GPIIIa polymorphism [37–39], and atherosclerosis. Studies of twins suggested a familial risk of coronary artery disease correlated with genetic factors, not merely a common familial environment [40]. To our knowledge, there are no reports of the relationship between genetics and cardiovascular diseases in Okinawa. Further studies will be needed to clarify this issue.

In conclusion, carotid IMT was low in I city. This is possibly related to the low rate of death due to cardiovascular disease and to residents of Okinawa having the highest longevity in Japan. Lower LDL cholesterol and other factors such as lifestyle, diet, genetics, climate and the environment in Okinawa may also be related to this phenomenon. Further research in Okinawa is necessary for the prevention of cardiovascular disease and to clarify the risk factors.

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A DECREASE IN MOTHER-TO-CHILD TRANSMISSION OF HUMAN T LYMPHOTROPIC VIRUS TYPE I (HTLV-I) IN OKINAWA, JAPAN

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Abstract. To investigate the chronologic change of mother-to-child transmission of human T lymphotropic virus type I (HTLV-I) in Okinawa, Japan, the presence of antibody to HTLV-I was tested in 4,187 healthy residents between, 4,528 nursery school children, and 3,837 pregnant women between 1968 and 2000. The chronologic change of the feeding method and the length of the breast-feeding period among 1,117 healthy mothers from 1937 to 1995 were also obtained by interview. Age-adjusted prevalence of HTLV-I among healthy residents decreased from 9.1% in 1968–1970 to 7.8% in 1981–1984 and to 6.3% in 1996–1998. The crude prevalence of antibody to HTLV-I among healthy residents less than 20 years old decreased significantly from 4.6% in 1968–1970 to 0.1% in 1996–1998 ($P < 0.0001$). The prevalence of antibody to HTLV-I among nursery school children decreased significantly over the study period, from a high of 1.8% in 1984 to a low of 0.2% in 1998 ($P = 0.03$). The prevalence among pregnant women decreased significantly from 5.6% in 1989–1992 to 3.7% in 1997–2000 ($P = 0.0275$). Prior to 1967, all healthy mothers breast-fed their children. After 1968, the use of bottled and mixed milk (breast milk and bottled milk) increased, with bottled milk becoming predominant after 1990 (89%). The percentage of healthy mothers breast-feeding for more than one year significantly decreased from 68.3% in 1937–1947 to 0.4% in 1990–1995 ($P < 0.0001$). Infection with HTLV-I in Okinawa has decreased mainly due to a reduction in the number of mothers breast-feeding and a shortening of the breast-feeding period. However, because the mother-to-child transmission rate among non-breast-feeders decreased from 12.8% in 1986–1991 to 3.2% in 1995–1999, there may be other factors involved in the decrease in mother-to-child transmission.

INTRODUCTION

The human T lymphotropic virus type I (HTLV-I) is characterized by infection of helper T cells and is known to be the pathogenic agent of adult T cell leukemia/lymphoma (ATLL),[1] HTLV-I-associated myelopathy/tropical spastic paraparesis,[2,3] uveitis,[4] opportunistic lung infections,[5] infections with *Strongyloides stercoralis*, [6] and cancer of other organs.[7]

Three main routes of HTLV-I transmission are known. The first is mother-to-child transmission, mainly due to ingestion of breast milk: breast-feeding has been reported to be the predominant route.[8] Because of the high rate of mother-to-child transmission of HTLV-I among children breast-fed for 3–6 months, since 1989 we have advised HTLV-I carrier mothers to bottle feed their infants.[9] Sexual transmission, mainly from men to women, is the second most frequent route.[10] The third most prevalent route is blood transfusion, which includes HTLV-I-positive cellular components.[11,12] However, the importance of this route has decreased since the start of screening of blood products for antibodies to HTLV-I in Japan in November 1987.[13]

ATLL develops after a long incubation period, with an estimated lifetime risk of approximately 5% in individuals infected before the age of 20 years.[14] Because the prognosis for patients with ATLL is extremely poor,[15] the prevention of mother-to-child transmission of HTLV-I is of the utmost importance.

Infection with HTLV-I has a peculiar geographic distribution, with half of all ATL patients in Japan found in Kyushu and Okinawa in the southwestern part this country.[16] We have been doing epidemiologic surveys of HTLV-I in Okinawa since the discovery of HTLV-I.[17]

In the present study, to determine the chronologic change of HTLV-I prevalence and the reasons for this change in Okinawa, we surveyed the following five items: 1) prevalence of antibody to HTLV-I among healthy residents over the 30-year study period, 2) prevalence of antibody to HTLV-I among nursery school children over a 15-year period, 3) prevalence of antibody to HTLV-I and p40tax among pregnant women over a 15-year period, 4) the relationship between the feeding method and the seroconversion rate for antibody to HTLV-1 among children born to carrier mothers over a 13-year period, and 5) the feeding method among healthy residents.

MATERIALS AND METHODS

Study area. Ishigaki and Iriomote Islands are located in a remote area of Okinawa in the subtropical zone approximately 1,000 km south of the main islands of Japan, which lie in the temperate zone (Figure 1). This area studied is located in the southwestern part of Okinawa, close to Taiwan, and is highly endemic for both HTLV-I and hepatitis B virus (HBV).[17,18]

Subjects Healthy residents, nursery school children, pregnant women, and children born to HTLV-I-carrier mothers of this area were surveyed. Venous blood samples were taken and sera were separated and kept frozen at –20°C before being sent to our laboratory for testing. Fully informed consent was obtained from each study subject. When study subjects were less than 20 years old, informed consent was obtained from their parents. The study was reviewed and approved by the Ethics Committee of Kyushu University.

Healthy residents. Serum samples were collected from healthy individuals who were examined as part of a free health examination, which included determination of the presence of HBV markers. The examinations were announced by written notices distributed to all households. Serum sample collection was done three times: from 639 individuals from 1968 to 1970, from 1,382 from 1981 to 1984, and from 2,166 from 1996 to 1998. The data for 1968–1970 were

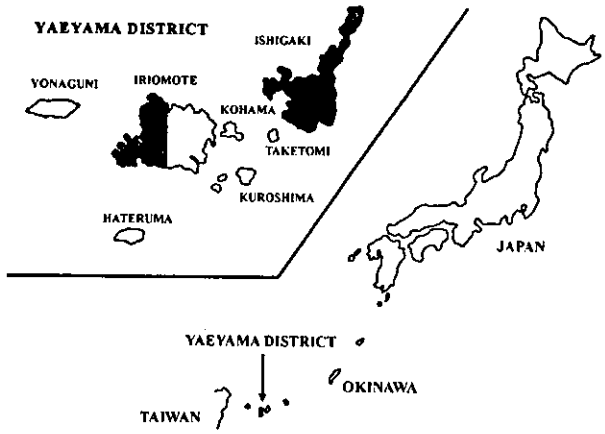

FIGURE 1. Location of the surveys for human T lymphotropic virus type I infection, Okinawa, Japan. The shaded areas indicate the areas surveyed. The map below the lines shows the survey area in relation to the main islands of Japan and Taiwan.

obtained by testing stored serum samples because they were obtained before the discovery of HTLV-I. The data for 1996–1998 included the samples of nursery school children on Ishigaki Island. The samples were the same as used in our previous surveys of hepatitis A virus, HBV, hepatitis C virus, and *Chlamydia pneumoniae* infections in the same study area.[18–22] The study subjects, except pregnant women, had not been informed of their antibody status for HTLV-I because no treatment is available for HTLV-I carriers.

The mothers of these children were questioned about the feeding method and the duration of breast-feeding in 1987 and 1995, if applicable. Data were obtained regardless of antibody to HTLV-I.

Nursery school children. Nursery school children in this area have been surveyed for HBV infection since 1979 as part of a primary care program for preschool children.[23] A total of 2,506 serum samples obtained from nursery school children 1–4 years old between 1984 and 1999 were used in the present study. School administrators and teachers informed parents of these tests. Samples were not obtained from children who were absent from school or whose parents did not consent. Children less than 12 months of age were not included in this study because it has been reported that the titer of maternally derived antibody decreases exponentially in the first 3–6 months after birth and becomes seronegative at approximately 6–9 months after birth.[24]

Pregnant women and children born to HTLV-I carrier mothers. A total of 3,837 pregnant women in Ishigaki Island who visited obstetric hospitals from 1989 to 2000 were examined antibody to HTLV-I after informed consent was obtained. Samples obtained from seropositive women were also tested for antibody to p40tax. As an important point of primary care, since 1989 we have advised HTLV-I carrier mothers to bottle feed their babies. The children of these HTLV-I-seropositive mothers were tested for antibodies to HTLV-I. Detailed questions concerning the number of offspring, whether they had breast or bottle fed, and the duration of breast-feeding were asked. In the studied area, public health nurses interview the mothers regularly after birth, regardless of their status for antibody to HTLV-I, about the feeding method and duration of breast milk and record information in a 'mother-and-baby notebook', which is given to all pregnant women by the government. The data collected in this study could not have been from selective recall. We tested 76 HTLV-I carrier mothers (age range = 20–40 years, mean age = 30.9 years) and 175 children (age range = 1–19 years, mean age = 5.5 years, 83 males and 92 females) between 1989 and 1991 and 36 HTLV-I carrier mothers (age range = 22–43 years, mean age = 31.2 years) and 76 children (age range = 1–17 years, mean age = 4.5 years, 34 males and 42 females) between 1995 and 1999.[9] No significant difference was found in the age of children born to carrier mothers when tested for antibody to HTLV-I between the two study periods. None of the children tested were married or had a history of blood transfusion or surgery. Children less than 12 months of age were not included for the reasons previously stated. There is a possibility that some aspect of the parent-child relationship other than feeding method influenced the status of antibody to HTLV-I of the child because there was more than one child born to some woman. However, it would be impossible to choose one child born to each carrier woman because of resultant selection bias.

Detection of antibody to HTLV-I. Screening for antibody to HTLV-I was done using the passive particle agglutination method (PA) (model FP 151; Fujirebio, Inc., Tokyo, Japan) for all samples. Samples positive by PA were confirmed by enzyme-linked immunosorbent assay (ELISA) (Eitest-ATL; Eisai, Tokyo, Japan) and by Western blot analysis (Eitest ATL-WB; Eisai) using antigens prepared from MT-2 cells.

TABLE 1

Age- and sex-specific and age-adjusted prevalence of anti-HTLV-I among healthy residents of Okinawa, Japan in the periods 1968–1970, 1981–1984, and 1996–1998*

	1968–1970			1981–1984			1996–1998		
Age (years)	No.	Positive (%)	AAP (%)	No.	Positive (%)	AAP (%)	No.	Positive (%)	AAP (%)
1–9	75	3 (4.0)		208	2 (1.0)		848	1 (0.1)	
10–19	121	6 (5.0)		205	5 (2.4)		91	0 (0)	
20–29	45	4 (8.9)		176	8 (4.5)		33	0 (0)	
30–39	82	9 (11.0)		199	16 (8.0)		123	3 (2.4)	
40–49	123	16 (13.0)		166	21 (12.7)		220	18 (8.2)	
50–59	108	14 (13.0)		195	25 (12.8)		129	22 (17.1)	
≥60	85	12 (14.1)		233	46 (19.7)		722	179 (24.8)	
Total	639	61 (9.5)	9.1	1,382	123 (8.9)	7.8	2,166	223 (10.3)	6.3

* Anti-HTLV-I = antibody to human T lymphotropic virus type I; AAP = age-adjusted prevalence. Age-adjusted prevalence was calculated using the direct standardization method with the 1985 Ishigaki city population as the standard population.

TABLE 2

Age-specific and age-adjusted prevalence of anti-HTLV-I among healthy male residents of Okinawa, Japan in the periods 1968–1970, 1981–1984, and 1996–1998*

Age (years)	1968–1970			1981–1984			1996–1998		
	No.	Positive (%)	AAP (%)	No.	Positive (%)	AAP (%)	No.	Positive (%)	AAP (%)
1–9	42	2 (4.8)		109	1 (0.9)		475	1 (0.2)	
10–19	64	2 (3.1)		100	1 (1.0)		47	0 (0)	
20–29	11	0 (0)		77	1 (1.3)		13	0 (0)	
30–39	29	3 (10.3)		106	6 (5.7)		45	1 (2.2)	
40–49	46	5 (10.9)		89	6 (6.7)		106	7 (6.6)	
50–59	58	8 (13.8)		93	14 (15.1)		59	8 (13.6)	
≥60	35	4 (11.4)		108	18 (16.7)		287	60 (20.9)	
Total	285	17 (6.0)	7.2	682	47 (6.9)	5.7	1,032	77 (7.5)	4.9

* Anti-HTLV-I = antibody to human T lymphotropic virus type I; AAP = age-adjusted prevalence. Age-adjusted prevalence was calculated using the direct standardization method with the 1985 Ishigaki city population as the standard population.

Since 1992, a new version of the ELISA has been used (New Eitest-ATL; Eisai, Tokyo, Japan). In the Western blot analysis, four proteins (p19, p24, p28, and gp68) were tested. Serum samples reactive to p19 plus at least two of the three remaining proteins were considered positive. Samples positive by all three methods were classed as positive for antibody to HTLV-I. Samples positive only by the PA test were considered negative, as were those positive by the PA test and ELISA but negative by Western blot analysis.

Detection of antibody to p40tax. Antibody to p40tax was measured by an ELISA using a recombinant p40tax protein expressed in *Escherichia coli* with a full length HTLV-I tax gene as the antigen. The cut-off value was set at the average of the p40tax ELISA absorbance value obtained from HTLV-I-seronegative specimens plus three standard deviations.[25]

Statistical analysis. The chi-square test, Fisher's exact test, and Mantel-Haenszel test were used for categorical variables for between group comparisons. A P value < 0.05 was considered significant. The Cochran-Armitage test for linear trends was used to analyze chronologic changes in the prevalence of antibody to HTLV-I and the relationship between duration of breast-feeding and the prevalence of antibody to HTLV-I. The age-adjusted prevalence of antibody to HTLV-I was calculated by direct standardization with the 1985 population of Ishigaki Island as the standard population (0–9 years old, total = 8,236, 4,238 males and 3,998 female; 10–19 years old, total = 6,777, 3,477 males and 3,300 females; 20–29 years old, total = 5,448, 2,712 males and 2,736 females; 30–39 years old, total = 6,693, 3,584 males and 3,109 females; 40–49 years old, total = 4,282, 2,254 males and 2,028 females; 50–59 years old, total = 4,256, 2,091 males and 2,165 females; ≥ 60 years old, total = 5,169, 2,218 males and 2,951 females).

RESULTS

The age-specific and age-adjusted prevalences (AAP) of antibody to HTLV-I in the Yaeyama district of Okinawa for the periods 1968–1970, 1981–1984, and 1996–1998 are shown in Tables 1–3 (Table 1 = total, Table 2 = males, and Table 3 = females). The AAP of antibody to HTLV-I decreased, although it was not statistically significant (from 9.1% in 1968–1970 to 7.8% in 1981–1984 and to 6.3% in 1996–1998). In each test period the prevalence of antibody to HTLV-I increased with age. The prevalence of antibody to HTLV-I was significantly higher in women than in men in 1981–1984 and in 1996–1998 (P = 0.001 and $P < 0.0001$, by Fisher's exact test). The prevalence of antibody to HTLV-I in children less than 20 years old decreased significantly: 9 (4.6%) of 196 in 1968–1970, 7 (1.7%) of 413 in 1981–1984, and 1 (0.1%) of 939 in 1996–1998 (1968–1970 versus 1996–1998; $P < 0.0001$ and 1981–1984 versus 1996–1998; P = 0.001, by Fisher's exact test). There was no significant difference between male and female healthy residents less than 20 years old.

The prevalence of antibody to HTLV-I among nursery school children is shown in Table 4. No significant difference was found between males and females in any year. The prevalence of antibodies to HTLV-I among nursery school children decreased significantly from 1.8% in 1984 to 0.2% in 1998 (P = 0.0103, by Fisher's exact test). The prevalence of these

TABLE 3

Age-specific and age-adjusted prevalence of anti-HTLV-I among healthy female residents of Okinawa, Japan in the periods 1968–1970, 1981–1984, and 1996–1998*

Age (years)	1968–1970			1981–1984			1996–1998		
	No.	Positive (%)	AAP (%)	No.	Positive (%)	AAP (%)	No.	Positive (%)	AAP (%)
1–9	33	1 (3.0)		99	1 (1.0)		373	0 (0)	
10–19	57	4 (7.0)		105	4 (3.8)		44	0 (0)	
20–29	34	4 (11.8)		99	7 (7.1)		20	0 (0)	
30–39	53	6 (11.3)		93	10 (10.8)		78	2 (2.6)	
40–49	77	11 (14.3)		77	15 (19.5)		114	11 (9.6)	
50–59	50	6 (12.0)		102	11 (10.8)		70	14 (20.0)	
≥60	50	8 (16.0)		125	28 (22.4)		435	121 (27.8)	
Total	354	39 (11.0)	10.1	700	76 (10.9)	9.9	1,134	148 (13.1)	7.7

* Anti-HTLV-I = antibody to human T lymphotropic virus type I; AAP = age-adjusted prevalence. Age-adjusted prevalence was calculated using the direct standardization method with the 1985 Ishigaki city population as the standard population.

TABLE 4
Sex-specific prevalence of anti-HTLV-I among nursery school children on Ishigaki Island, Okinawa, Japan from 1984 to 1999*

Year	Male		Female		Total	
	No.	Positive (%)	No.	Positive (%)	No. tested	Positive (%)†
1984	154	2 (1.3)	127	3 (2.4)	281	5 (1.8)
1985	203	2 (1.0)	176	1 (0.6)	379	3 (0.8)
1986	609	3 (0.5)	504	7 (1.4)	1,113	10 (0.9)
1994	431	1 (0.2)	368	1 (0.3)	799	2 (0.3)
1996	279	1 (0.4)	230	1 (0.4)	509	2 (0.4)
1998	392	1 (0.3)	272	0 (0)	664	1 (0.2)
1999	438	3 (0.7)	344	2 (0.6)	782	5 (0.6)
Total	2,506	13 (0.5)	2,021	15 (0.7)	4,527	28 (0.6)

* Anti-HTLV-I = antibody to human T-lymphotropic virus type I.
† $P = 0.0112$, by Cochran-Armitage test for linear trends.

antibodies among nursery school children decreased significantly over the study period ($P = 0.0277$, by Cochran-Armitage test for linear trends).

The prevalence of antibodies to HTLV-I among pregnant woman and their serostatus for antibodies to p40 tax in Table 5. The overall prevalence of antibodies to HTLV-I among pregnant women was 4.6% (177 of 3,837). This prevalence significantly decreased over the study period: 5.6% (76 of 1,362) in 1989–1992, 4.4% (61 of 1,387) in 1993–1996, and 3.7% (40 of 1,088) in 1997–2000 (1989–1992 versus 1997–2000; $P = 0.0275$, by chi-square test). Of the 177 pregnant women positive for antibody to HTLV-I, 8 (46.3%) were positive for antibody to p40tax. The percentage of patients positive for p40tax fluctuated: 50% (38 of 76) in 1989–1992, 37.7% (23 of 61) in 1993–1996, and (52.5%) 21 of 40 in 1997–2000. No significant difference was found in the percentage of patients positive for p40tax during the three periods.

The periods of breast-feeding and HTLV-I infection among children born to carrier mothers on Ishigaki Island from 1986 to 1991 and from 1995 to 1999[9] are shown in Table 6. The rate of non-breast-feeding or breast-feeding ≤ 3 months among breast-feeders increased from 66.9% (117 of 175) in 1986–1991 to 73.7% (56 of 76) in 1995–1999, although it failed to reach statistical significance. The prevalence antibodies to HTLV-I among children born to carrier mothers decreased significantly from 16.0% (28 of 175) in 1986–1991 to 3.9% (3 of 76) in 1995–1999 ($P = 0.0063$, by Fisher's exact test). In 1986–1991, as the duration of breast-feeding increased, the prevalence of antibody to HTLV-I among children born to carrier mothers increased significantly ($P = 0.0066$, by Cochran-Armitage test for linear trends). The rate of mother-to-child infection among non-breast-feeders also decreased from 12.8% (10 of 78) in 1986–1991 to 3.2% (1 of 31) in 1995–1999. The overall rate of mother-to-child infection among breast-feeders decreased significantly from 18.6% in 1986–1991 to 4.4% in 1995–1999 ($P = 0.0355$, by Fisher's exact test). The positivity rates for antibody to HTLV-I in all categories of breast-feeding (≤ 3 months, ≤ 6 months, ≤ 12 months, and > 12 months) decreased from the period 1986–1991 to 1995–1999 (5.1% versus 4.0%, 22.7% versus 0%, 26.1% versus 0%, and 38.5% versus 11.1%, respectively).

The chronologic change of the feeding method and the length of the breast-feeding period among healthy mothers on Ishigaki Island from 1937 to 1995 are shown in Table 7. Data were obtained by interviewing healthy mothers in 1987 and 1995. Prior to 1967, all mothers breast-fed their children. After 1968, the use of bottled and mixed milk (breast milk and bottled milk) increased, with bottled milk becoming predominant after 1990 (400 of 450 [89%]). The percentage of healthy mothers breast-feeding for more than one year decreased significantly from 68.3% in 1937–1947 to 0.4% in 1990–1995 ($P < 0.0001$, by Fisher's exact test).

TABLE 5
Prevalence of anti-HTLV-I and anti-p40tax among pregnant women on Ishigaki Island, Okinawa, Japan from 1989 to 2000*

Years	Anti-HTLV-I		Anti-p40tax†	
	No.	Positive (%)	No.	Positive (%)
1989–1992	1,362	76 (5.6)‡	76	38 (50.0)
1993–1996	1,387	61 (4.4)	61	23 (37.7)
1997–2000	1,088	40 (3.7)‡	40	21 (52.5)
Total	3,837	177 (4.6)	177	82 (46.3)

* Anti-HTLV-I = antibody to human T-lymphotropic virus type I; Anti-p40tax = antibody to p40tax.
† Anti-p40tax was tested for in all anti-HTLV-I–positive subjects.
‡ $P = 0.0275$, by chi-square test.

DISCUSSION

Other population-based studies have reported prevalences of antibody to HTLV-I ranging from 3–6% in Jamaica, Trinidad, and the Caribbean Islands to 23.2% in Nagasaki Prefecture in southwestern Japan.[26–28] In the present study, although the prevalence of HTLV-I decreased chronologically from 1968–1970 to 1996–1998, Okinawa remains a highly

TABLE 6
Period of breast-feeding and HTLV-I infection among children born to carrier mothers in the periods 1986–1991 and 1995–1999 on Ishigaki Island, Okinawa, Japan*

Period of breast-feeding	1986–1991		1995–1999	
	No.	Positive (%)	No.	Positive (%)
Non-breast-feeding	78	10 (12.8)	31	1 (3.2)
Breast-feeding	97	18 (18.6)†	45	2 (4.4)†
≤3 months	39	2 (5.1)	25	1 (4.0)
≤6 months	22	5 (22.7)	4	0 (0)
≤12 months	23	6 (26.1)	7	0 (0)
>12 months	13	5 (38.5)	9	1 (11.1)
Total	175	28 (16.0)‡	76	3 (3.9)‡

* Anti-HTLV-I = antibody to human T-lymphotropic virus type I.
† $P = 0.0355$, by Fisher's exact test.
‡ $P = 0.0063$, by Fisher's exact test.

TABLE 7

Chronological change of feeding method and the length of the breast-feeding period among healthy mothers on Ishigaki Island, Okinawa, Japan, from 1937 to 1995*

Birth year	1937–1947	1948–1967	1968–1983	1990–1995
Breast milk				
Over 1 year	56 (68.3%)	136 (39.0%)	16 (11.8%)	2 (0.4%)
Less than 1 year	22 (26.8%)	188 (53.9%)	59 (43.4%)	48 (10.7%)
Mixed milk	4 (4.9%)	25 (7.2%)	54 (39.7%)	0
Bottled milk	0	0	7 (5.1%)	400 (88.9%)
Total	82 (100%)	349 (100%)	136 (100%)	450 (100%)

* Data were obtained by interview in 1987 and 1995. Mixed milk = combined use of breast milk and bottled milk.

HTLV-I-endemic area. In a follow-up study of residents negative for HTLV-I in the 1980s, few new cases of HTLV-I transmission in the younger age groups were found after a nine-year interval.[29] Therefore, we concluded that higher prevalence rates of antibody to HTLV-I in adults reflects previous higher rates of transmission as infants. The present long-term observations confirmed our previous results.

In each study period, the prevalence of antibody to HTLV-I after the age of 20 years was always higher in women than in men. We previously reported that the man-to-woman transmission rate of married couples was 60%/10 years, in contrast to a woman-to-man transmission rate of 0.4%/10 years.[10] The difference in the prevalence of antibody to HTLV-I between men and women probably reflected man-to-woman transmission in the sexually active ages. No sex difference was seen in the prevalence of antibody to HTLV-I among nursery school children, who are not at a sexually active age.

The significant chronologic decrease in the prevalence of antibody to HTLV-I among healthy residents less than 20 years old, including nursery school children, indicates that mother-to-child infection or infection in childhood has decreased. However, the prevalence of antibody to HTLV-I in 1999 increased slightly. We can not clarify why the prevalence of this antibody had increased. Since the prevalence of antibody to HTLV-I after 1994 was so low (< 1.0%), it was difficult to evaluate the annual change in the prevalence of this antibody. A follow-up study will be needed to clarify this matter. Mother-to-child transmission rates among breast-feeders have been reported to range from 5.7% to 39%.[8,30,31] We also reported that the mother-to-child transmission rate by breast-feeding was 18.6% in our study area in 1986–1991 and that the HTLV-I transmission rate increased with the duration of breast-feeding from HTLV-I carrier mothers.[9] The mother-to-child transmission rate among breast-feeders significantly decreased from 1986–1991 to 1995–1999 in Okinawa. Although maternal antibody to p40tax is a marker of relative infectivity in mother-to-child transmission,[25] its prevalence among HTLV-I carrier mothers did not change between 1986 and 2000. Therefore, it is reasonable to assume that the decrease in HTLV-I infection was, at least partly, caused by a shortening of the breast-feeding period.

The mother-to-child transmission rate among non-breast-feeders in this area in 1986–1991 (12.8%), which was higher than that of other HTLV-I-endemic areas in Japan (0.4–4.1%), also decreased in 1995–1999 (3.2%).[8,31] This decrease can not be explained by the change of feeding method. The mode of perinatal HTLV-I transmission was unclear except for breast-feeding. In follow-up studies of non-breast-feeders, mother-to-child transmission was not correlated with the status of antibody to HTLV-I in cord blood.[32] Bittencourt and others reported that a cesarean section protected against mother-to-child transmission of HTLV-I among non-breast-fed children.[33] Therefore, transplacental mother-to-child infection seems to be rare. Since 1991, when we begun educating doctors about this route and advised them to protect against nosocomial infection, obstetricians and gynecologists in this study area have taken precautions to ensure that newborns do not swallow blood at the time of delivery. Their efforts may also have contributed to the relative decrease in the transmission rate of HTLV-I from 12.8% to 3.2% in the present study.

Infection with HTLV-I in Okinawa seems to have decreased mainly due to a reduction in the number of mothers breast-feeding and a shortening of the breast-feeding period. In addition, mother-to-child transmission among non-breast-feeders also decreased, indicating that there may be other factors that led to the decrease in mother-to-child transmission.

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Short-term Interferon-alfa Therapy for Acute Hepatitis C: A Randomized Controlled Trial

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Acute hepatitis C often progresses to chronic infection. We undertook a randomized controlled trial to determine whether short-term therapy with interferon (IFN) during acute hepatitis C is effective in preventing the development of chronic hepatitis. Thirty patients with acute hepatitis C were randomized into 1 of 2 treatment groups. IFN therapy was initiated 8 weeks after the onset of acute hepatitis in the early-intervention group and after 1 year of observation in the late-intervention group. Short-term therapy consisted of natural IFN-alfa (6 million units) administered on consecutive days for a period of 4 weeks. Any signs of recrudescence of disease were immediately followed by interval IFN therapy (3 times weekly for 20 weeks). In the early-intervention group, short-term therapy was associated with a sustained virological response in 13 of 15 patients (87%). Follow-up treatment was associated with a sustained virological response in both of the remaining 2 patients (100%). The sustained virological response rate was significantly higher in the early-intervention group (87%, 13 of 15 patients after short-term therapy alone, and 100%, 15 of 15 patients after short-term with or without follow-up therapy) than in the late-intervention group (40%, 6 of 15 patients after short-term therapy alone, and 53%, 8 of 15 patients after short-term therapy with or without follow-up therapy, $P = .021$ and $P = .006$, respectively). In conclusion, short-term (4 weeks) IFN treatment of patients with acute hepatitis C may be associated with satisfactory results, if initiated at an early stage of the disease. (HEPATOLOGY 2004;39:1213–1219.)

Acute hepatitis that develops after infection with the hepatitis C virus (HCV) is often followed by chronic hepatitis, which may progress eventually to cirrhosis and hepatocellular carcinoma (HCC).[1,2] In the past, the primary causes of infection with HCV were blood transfusion and various medical procedures. Today, blood products in Japan are aggressively screened for HCV and disposable medical devices are in widespread use; there has been a reduction in the incidence of HCV infection. However, patients with acute hepatitis C resulting from treatment-related accidents (needle-stick injury), intravenous drug abuse, sexual contact with HCV-positive partners and unknown causes still occasionally present.[3–5] Interferon (IFN) therapy in patients with chronic hepatitis C has considerable potential for preventing the development of HCC, either by eradicating HCV, or by decreasing the activity of hepatitis.[6–9] However, the therapeutic effects of IFN vary depending on the HCV genotype and viral load.[10,11]

Although much research has already been undertaken on IFN therapy for acute hepatitis C, findings in trials that relate to the effectiveness of this therapy have not been particularly favorable. Possible reasons include differences in types of IFN, differences in study populations, and inclusion of patients with posttransfusion hepatitis.[12–17] However, Jaeckel et al.[3] reported that a 24-week course of IFN therapy was effective, and that the response to IFN treatment was more favorable in acute hepatitis C than in chronic hepatitis C. Although randomized controlled trials have been used to study the effects of IFN therapy on acute hepatitis C, so far there have been no

Abbreviations: HCV, hepatitis C virus; HCC, hepatocellular carcinoma; IFN, interferon; ALT, alanine aminotransferase; ULN, upper limit of the normal range; MU, million units; HLBI, human lymphoblastoid interferon.

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reports on the most suitable duration of IFN treatment or timing of its initiation.

Short-term (4 weeks) IFN therapy was administered at an early stage of acute hepatitis in patients who met strict diagnostic criteria for acute hepatitis C. The objective was to corroborate the effectiveness of short-term IFN therapy in the treatment of this condition. A randomized controlled trial was designed to determine the appropriate duration of treatment and the timing of initiation of treatment with IFN therapy for acute hepatitis C. The results indicated that it should not be necessary to treat all patients with acute hepatitis C with the 24-week course of treatment that is most commonly administered for chronic hepatitis C in Japan.

Patients and Methods

Patients. Thirty-nine patients with acute hepatitis C who attended Shin-Kokura Hospital between January 1994, and December 2000, were studied. Criteria for the diagnosis of acute hepatitis C were: (1) At the onset of acute hepatitis, serum alanine aminotransferase (ALT) levels at least 7 × upper limit of the normal range (ULN), HCV-RNA positive and anti-HCV negative; or (2) negative HCV-RNA at the time of a needle-stick accident, but subsequent HCV-RNA positive and ALT levels at least 7 × ULN. All of the patients with acute hepatitis C had no history of blood transfusion. The patients were negative for both immunoglobulin M anti–hepatitis A virus and antinuclear antibody. Patients infected with the human immunodeficiency virus were not included in the study. Two patients were hepatitis B surface antigen positive, and 3 patients had been drinking at least 80 g of alcohol daily; these 5 patients were excluded from the study. At the onset of acute hepatitis, 20 of the remaining 34 patients had symptoms such as general malaise, loss of appetite, and a feeling of abdominal fullness; 14 patients were asymptomatic. The 20 symptomatic patients were diagnosed with acute hepatitis C when they developed symptoms. In 8 of the 14 asymptomatic patients, the diagnosis was made during follow-up after a needle-stick injury. Of the 10 patients followed after needle-stick injury, 8 were asymptomatic and 2 developed symptoms. The remaining 6 asymptomatic patients were diagnosed with acute hepatitis C, as a result of regular tests conducted during clinic visits for other diseases, such as diabetes mellitus.

For 8 weeks after developing acute hepatitis C, 34 patients were tested once weekly for serum ALT and once monthly for HCV-RNA. Of these, 4 patients cleared HCV-RNA during the 8 weeks of initial observation. In 3 of 20 patients with symptoms at onset, clearance of HCV-RNA occurred within 8 weeks. Total bilirubin increased to levels greater than 3.0 mg/dL in 3 of 34 patients during the 8-week follow-up period. In 2 of these 3 patients with jaundice, HCV-RNA clearance occurred within 8 weeks. One patient was positive for HCV-RNA after 8 weeks and was treated with IFN. A total of 30 patients, who were HCV-RNA positive and had ALT levels of at least 7 × ULN at 8 weeks, were enrolled in the study. The route of infection was needle-stick accident in 10 patients, intravenous drug use in 3 patients, sexual contact with an infected partner in 4 patients, and unknown in 13 patients. The time from exposure to onset of hepatitis was 7.3 ± 2.1 weeks (mean ± SD) (range, 5-12 weeks) in 10 the patients who had had a needle-stick injury and who were followed from the time of infection until the time symptoms appeared. Of the 5 patients in the early-intervention group, the time from infection to the start of treatment was 15.6 × 2.7 (range, 13-20) weeks.

Thirty patients were randomized at enrollment into 1 of 2 treatment groups using an enrollment sheet method; there were 15 patients per group. IFN therapy was initiated 8 weeks after acute hepatitis was diagnosed in the early-intervention group, and after 1 year of follow-up in the late-intervention group. The initial plan in this study, which was designed in 1994, was to recruit 30 patients. Characteristics of the patients studied are shown in Table 1. The patients included 19 men and 11 women, whose ages ranged from 22 to 59 years; the mean age was 40.4 years in the early-intervention group and 37.6 years in the late-intervention group. There was no significant difference in sex ratio and age between the two groups. Viral

Table 1. Patient Baseline Characteristics

	Early-Intervention Group N = 15	Late-Intervention Group N = 15	Total N = 30
Age (y, mean ± SD)	40 ± 11	38 ± 10	39 ± 10
Gender (male/female)	10/5	9/6	19/11
Baseline test values (before IFN therapy)			
Serum ALT (IU/L, mean ± SD)	491 ± 181	431 ± 143	460 ± 161
HCV-RNA (low/high)*	8/7	9/6	17/13
(10^5 copies/mL, mean ± SD)†	2.91 ± 2.41	2.56 ± 2.78	2.74 ± 2.66
Genotype (1b/others)	12/3	13/2	25/5
Mode of HCV Infection			
Needle-stick injury	5	5	10
Intravenous drug use	1	2	3
Sexual contact with HCV-positive partners	2	2	4
Unclear	7	6	13

Abbreviation: y, years.
*HCV load: low (<1 × 10^5 copies/mL), high (≥ 1 × 10^5 copies/mL)
†Copies/mL refers to HCV-RNA.

load was measured immediately before IFN therapy, using an Amplicor-HCV monitor assay (Roche Molecular Diag., Tokyo, Japan). Patients were designated as having a low viral load (less than 10^5 copies/mL), or a high viral load (10^5 copies/mL or greater). There was no significant difference in viral load between the early-intervention group and the late-intervention group. The genotype of HCV was 1b in about 80% of patients; there was no significant difference in the frequency of this genotype between the two groups. The ULN for serum ALT was set at 40 IU/L.

Protocol for Interferon Treatment

Initial Interferon Therapy (Short-term Interferon Therapy). Patients in the early-intervention group were treated with 6 million units (MU) of natural IFN-alfa (human lymphoblastoid interferon [HLBI], Sumitomo Pharmaceutical, Osaka, Japan) by intramuscular injection, once daily for 4 consecutive weeks during the early stage of acute hepatitis. The clinical course of patients in the late-intervention group was monitored for 1 year; patients continuing to be HCV-RNA positive after 1 year were treated with 6 MU of HLBI by intramuscular injection once daily for a period of 4 consecutive weeks.

Follow-up Interferon Therapy. Following completion of 4 the initial weeks of IFN therapy, all patients were followed-up, and HCV-RNA and serum ALT were monitored. Additional IFN therapy was administered to patients who were HCV-RNA positive after completion of the initial course of IFN therapy or who relapsed and became HCV-RNA positive again after completion of the initial course of IFN therapy. IFN therapy was 6 MU of HLBI injected intramuscularly, 3 times weekly, for 20 weeks. For all patients, IFN therapy was administered at Shin-Kokura Hospital.

Serological and Virological Assays. Serum ALT levels were measured once weekly during the initial 4-week period of treatment, and once every 4 weeks during follow-up IFN therapy and monitoring after treatment. During initial treatment, HCV-RNA was measured at Week 1, Week 2, and upon completion of treatment. In addition, these measurements were also undertaken at 4, 8, 12, 16, 20, and 24 weeks after completion of the initial course of treatment or follow-up IFN therapy. Anti-HCV antibodies were determined at the onset of hepatitis and 24 weeks after completion of IFN therapy, using a second-generation enzyme immunoassay. Effective IFN therapy was considered to be the induction of a sustained virological response—that is, HCV-RNA remaining negative for 24 weeks after completion of the initial course or the follow-up course of IFN therapy. HCV-RNA was measured using 2 methods, nested polymerase chain reaction and Amplicor-HCV assay, version 2.0 (Roche Molecular Diag., Tokyo, Japan), 24 weeks after completion of the initial or follow-up course of IFN therapy. Negative results of both tests were required to infer a sustained virological response. Those patients who were not classified as having undergone a sustained virological response were considered to be nonresponsive. Patients who underwent a sustained virological response were followed for at least 2 years after treatment, and HCV-RNA was measured again at the end of the 2-year period of follow-up, using the Amplicor-HCV assay, version 2.0. HCV genotyping was undertaken as previously described.[18]

Informed Consent. The study protocol was approved by institutional ethics committees. All patients gave written informed consent to participate in this study. The study was conducted in accordance with ethical guidelines of the Declaration of Helsinki and the International Conference on Harmonization guidelines for good clinical practice. No patient withdrew informed consent to participate in the study.

Statistical Analysis. The early-intervention and late-intervention groups, and the sustained virological response and nonresponsive groups were compared using the unpaired Student *t* test or Fisher exact test. All *P* values reported in this study were 2-tailed. *P* values less than .05 were considered to be statistically significant.

Results

Effect of Early- vs. Late-Intervention IFN Therapy. Results of IFN therapy are shown in Table 2. A sustained virological response occurred in 13 out of 15 (87%) patients in the early-intervention group following initial therapy. A recrudescence of disease developed in 2 patients in the early-intervention group 4 weeks after completion of the initial therapy. In these 2 patients a sustained virological response occurred after a 20-week course of follow-up IFN therapy. Thus, sustained virological responses occurred in all 15 patients in this group. In the late-intervention group, serum ALT levels fell below 2 × ULN in 6 patients during the 1-year monitoring period, but all patients were HCV-RNA positive immediately before IFN therapy. A sustained virological response occurred after initial IFN therapy in 6 (40%) patients in this group; a recrudescence of disease occurred in 9 patients. These 9 patients received follow-up IFN therapy, and a sustained virological response subsequently occurred in 2 of them. Thus, 8 of 15 (53%) patients in the late-intervention group had a sustained virological response. The sustained virological response rate was significantly higher in the early-intervention group than in the