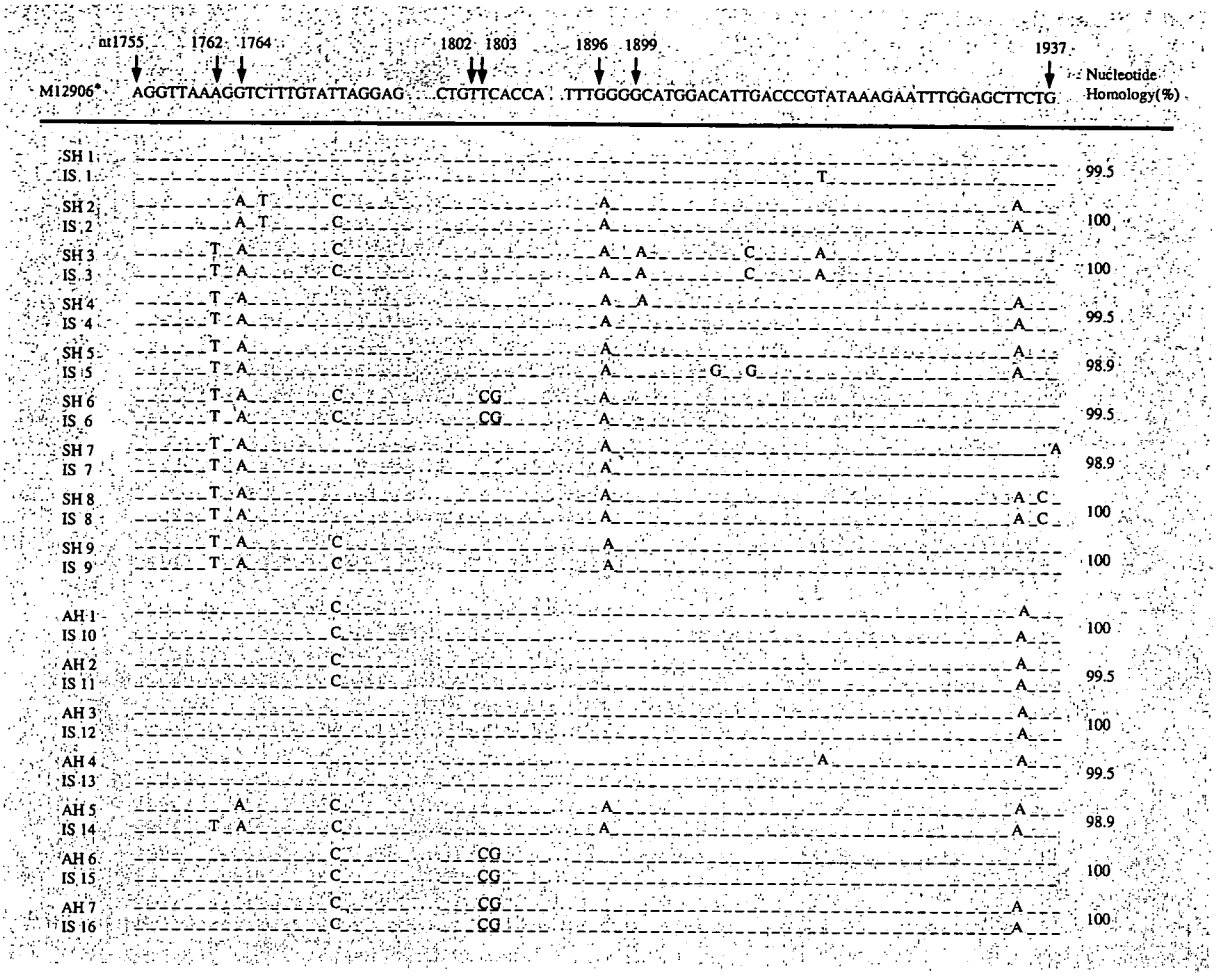

Fig. 1. Comparison of HBV nucleotide sequence in patients with acute HBV infection and their infectious sources. SH = Severe acute hepatitis; AH = typical acute hepatitis; IS = infectious source. * From Kobayashi and Koike [49].

Results

Features of Patients with Acute HBV Infection

Sixteen patients with acute HBV infection were infected with genotype C, 2 with genotype D and 1 with genotype A. Table 1 shows clinical features of 16 patients with acute HBV genotype C infection, with data from the early stage of onset of hepatitis. There were no differences for age and sex distribution between the SH and AH groups. One patient with fulminant hepatitis (SH2) died, 1 with fulminant hepatitis (SH3) received a liver transplant and the other 14 were alive at the time of this study. Among those with genotype C HBV infection, core promoter mutations (nt 1762T, nt 1764A) and a precore mutation (nt 1896A) were significantly higher in patients with SH (7/9 vs. 0/7, $p < 0.05$; 8/9 vs. 1/7, $p < 0.01$; 8/9 vs. 1/7, $p < 0.01$, respectively).

Features of Infectious Sources

Among all 19 infectious sources, 2 were infected with genotype D, 1 had genotype A and 16 had genotype C. Five of them were spouses and 14 were sexual partners. The HBV genotype was identical in all 19 pairs. Table 2 shows data of the infectious source subjects with genotype

Table 3. The comparison of clinical features of infectious sources of patients with acute genotype C HBV infection[a]

	Infectious sources of patients with		p value
	SH	AH	
Number	9	7	
Age, median (range), years	40 (21–57)	22 (19–39)	0.037
Sex, M/F	5/4	2/5	0.358
Diagnosis			0.035
ASC	1	5	
CH, LC, HCC	8	2	
T. bil			0.999
Within normal range, < 1.2 mg/dl	8	7	
Elevated ≥ 1.2 mg/dl	1	0	
ALT			0.041
Within normal range (<40 IU/l)	2	6	
Elevated (≥ 40 IU/l)	7	1	
HBeAg/anti-HBe state			0.009
HBeAg +	1	6	
Anti-HBe +	8	1	
Mutation in core promoter region			
nt 1762T	7	1	0.041
nt 1764A	8	1	0.009
Mutation in precore region			
nt 1896A	8	1	0.009

[a] ASC = Asymptomatic carrier; CH = chronic hepatitis; LC = liver cirrhosis; HCC = hepatocellular carcinoma; T. bil = total bilirubin; ALT = alanine aminotransferase; SH = severe acute hepatitis; AH = typical acute hepatitis.

C infection. Among the 9 sources of SH, 1 was diagnosed as an asymptomatic carrier and the other 8 had chronic liver diseases (5 with chronic hepatitis, 2 with liver cirrhosis, 1 with hepatocellular carcinoma). Of the 7 sources of AH, 5 were asymptomatic carriers and 2 had chronic hepatitis.

Comparison of HBV between Partner Pairs

Nucleotide sequences of all subjects infected with genotype C between nt 1755 and nt 1937 (183 bases) were analyzed, and their sequences are partially shown in figure 1. A comparison of the nucleotide sequences revealed that the nucleotide homology between each pair was ≥98.9%, and the same nucleotide substitutions at the same positions were seen in each pair. Therefore, the sexual partners were confirmed as the respective infectious sources.

Features of Infectious Sources Infected with Genotype C

A comparison of clinical features between 9 infectious sources of SH and 7 infectious sources of AH is shown in table 3. Statistical differences were observed for age, diagnosis, ALT, HBeAg/Ab and mutations at nt 1762, nt 1764 and nt 1896. Age ($p < 0.05$), ratio of patients with chronic liver diseases ($p < 0.05$), elevated ALT ($p < 0.05$), anti-HBe ($p < 0.01$) and the mutations at nt 1762T ($p < 0.05$), nt 1764A ($p < 0.01$) and nt 1896A ($p < 0.01$) were significantly higher in the infectious sources of SH than in those of AH. Among 6 of the infectious sources diagnosed as asymptomatic carriers, 1 was a source of SH and positive for anti-HBe, while the other 5 were infectious sources of AH and positive for HBeAg. Multivariate analysis was not possible in this study because the number of patients was restricted.

Discussion

Previous studies concerning factors related to the severity of acute HBV infection were mainly performed by analyzing virological aspects of patients. Many reports, mainly from Asia, have shown that the G to A mutation at nt 1896 in the precore region that induces translational

stop codon, and the A to T mutation at nt 1762 and the G to A mutation at nt 1764 in the core promoter region are frequently found in patients with fulminant hepatitis [16–21]. These mutations in the precore and core promoter regions are related to a reduction of the HBeAg by the translational and transcriptional levels, respectively. The mechanisms involved with severe liver damage and these mutations are under investigation in relation to viral replication, viral gene expression and localization of viral proteins [22–27]. On the other hand, there are many reports from western countries showing that the frequencies of these mutations were not high among fulminant hepatitis patients and that they were not related to the severity of hepatitis [28–32]. It has been reported that the frequencies of these mutations are different among HBV genotypes [33–36]. The differences of frequency of these mutations in fulminant hepatitis in western and eastern countries may be related to the difference of HBV genotype distribution throughout the world, as genotypes B and C are major genotypes in East Asia, and genotypes A and D are major in the US and Europe [33, 34, 37–39].

Several investigators have studied infectious source factors in cases of infant patients with fulminant hepatitis B, and some results have indicated that anti-HBe positivity or mutant HBV with a precore mutation (nt 1896G to A) in the mother has a relation with fulminant hepatitis in the child; however, some reports are contradictory [40–44]. In cases of adult patients with acute HBV infection, only a few reports have discussed the virological aspects of the infectious sources. Aye et al. [45] studied 7 adult patients with acute HBV infection and 7 HBV carriers who were their infectious sources, and found that all 5 infectious sources with acute hepatitis B were positive for HBeAg without precore mutant HBV and 4 of the 5 were healthy carriers, whereas 2 of 2 infectious sources of fulminant hepatitis were patients with chronic hepatitis and 1 was positive for anti-HBe with precore mutated HBV. Further, Yotsumoto et al. [46] and Tanaka et al. [47] noted that infectious sources of adult patients with fulminant hepatitis were positive for anti-HBe with a precore mutation. However, there are no known reports that have performed statistical analysis of the risk factors in infectious sources in relationship to the severity of hepatitis in partner patients.

In the present study, we analyzed the clinical features and viral genomes of infectious sources infected with genotype C HBV, which is the main genotype found in our region. It has been proven that the infectious source factors that related to the transmission of SH were higher age, anti-HBe, chronic liver diseases, ALT abnormality and mutations in core promoter and precore regions. It is interesting that all of these factors were related to the immune pressure to HBV in the chronically infected host. Therefore, it is suspected that HBV strains that are able to replicate under host immune pressure may have a relationship to SH in the receivers. HBeAg-positive asymptomatic carriers are thought to be at the immune tolerance stage in the course of chronic HBV infection. All patients infected by an HBeAg-positive asymptomatic carrier were diagnosed with AH, and no infectious sources with SH were diagnosed as an HBeAg-positive asymptomatic carrier. One infectious source of SH was diagnosed as ASC; however, this source was positive for anti-HBe, and it may be better to diagnose this case as an inactive HBsAg carrier state [48]. These results also support the suspicion that replication-competent HBV strains under immune pressure have a relation with SH in receivers. These results of adult acute HBV infection were not inconsistent with reports of infant cases with fulminant hepatitis B, suggesting the importance of anti-HBe and the precore mutant in mothers.

The present results confirmed that core promoter or precore mutations are related to the severity of acute genotype C HBV infection, as seen from the analyses of HBV in patients with acute HBV infection and their infectious sources. However, all but one infectious sources in this study were infected with either mutant type HBV (nt 1762T, nt 1764A and nt 1896A) or wild type HBV (nt 1762A, nt 1764G and nt 1896G); therefore, it was not possible to analyze and compare the impact of the nature of these 3 mutations with the severity of hepatitis in infected patients.

Because of the restricted number of patients in this study, statistical analysis could only be done for genotype C. Risk factors relating to the severity of hepatitis might be different among different genotypes. This issue should be clarified in the future.

In conclusion, among the partners of patients chronically infected with genotype C HBV, those who were diagnosed with chronic liver diseases, positive for anti-HBe and infected with core promoter (nt 1762T, nt 1764A) or precore (nt 1986A) mutants were identified as risky infectious sources of sexually transmitted severe hepatitis.

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Journal of Gastroenterology

Risk factors for development of hepatocellular carcinoma in patients with chronic hepatitis C after sustained response to interferon

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Editorial on page 220

Background. Interferon (IFN) is expected to prevent the progression of hepatitis C virus infection to cirrhosis and the development of hepatocellular carcinoma (HCC), but there have been several reports of the development of HCC after a sustained response to IFN. Our aim was to elucidate the incidence and clinical features of, and risk factors for, HCC in sustained responders to IFN, taken for the treatment of chronic hepatitis C. ***Methods.*** We designed a retrospective cohort study conducted at 16 major Hospitals. The subjects were a total of 1056 patients showing sustained responses, 29 of whom developed HCC. ***Results.*** The incidence of HCC per 100 person-years was 0.56 (95% confidence interval, 0.35–0.76) in sustained responders. By the Cox proportional hazard model, we found that older age, higher serum aspartate aminotransferase level, and lower platelet count before IFN therapy were independent risk factors associated with the development of HCC. A risk index of HCC development, based on the coefficients of these risk factors, was used to classify patients into three groups, with low, intermediate, and high risk. The incidence rates of HCC for these three groups were 0.11, 0.44, and 1.98 per 100 person-years, respectively. The median period to the development of HCC was 4.6 years (range, 1.4–9.0 years), and there were no other specific clinical features of the HCC that developed in these patients. ***Conclusions.*** This study suggests that the risk of development of HCC is not completely eliminated in sustained responders to IFN. These findings may be useful in determining a follow-up strategy after a sustained response to IFN.

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Key words: hepatitis C virus, hepatocellular carcinoma, interferon, sustained response

Introduction

Hepatitis C virus (HCV) infection is one of the most common causes of chronic hepatitis, and it is also a major risk factor for hepatocellular carcinoma (HCC).[1,2] Chronic hepatitis C is often asymptomatic and mild, but may slowly progress to liver cirrhosis and eventually to HCC.[3–5] Therefore, it has been assumed that eradication of HCV would provide the most effective means of preventing HCC.

Currently, interferon (IFN) represents the mainstay of treatment for chronic hepatitis C.[5–9] IFN therapy can lead to a decrease in serum transaminase activity, and to the disappearance of serum HCV RNA in patients with chronic hepatitis C. These patients appear to benefit by the prevention of progression to cirrhosis and HCC.[5,7,10–14] However, HCC can still occur in patients who are treated successfully with IFN, i.e., those showing a sustained response to the therapy.[5,10–25] The incidence and clinical features of HCC, and the risk factors for carcinogenesis, have not yet been investigated, although they have been documented in individuals and in small numbers of patients.[5,10–25] We investigated a large cohort of patients showing a sustained response to IFN therapy given for chronic hepatitis C. Our aims were to assess the incidence of HCC in these patients and to discover the clinical variables that may be associated with the development of HCC. Our study also focused on the clinical features of HCC. We designed a multicenter retrospective cohort study, because a single-institution study would have provided inadequate numbers of sustained responders who developed HCC.

Patients and methods

Patients

This study was conducted at 16 major hospitals belonging to the Japanese Society of Gastroenterology, Kyushu Division. A large cohort of sustained responders to IFN therapy given for chronic hepatitis C, in whom HCC had, or had not, been detected, was assembled consecutively by means of data collection instruments. All sustained responders included in the study were positive for HCV RNA before IFN therapy, and were followed up for more than 1 year after termination of IFN therapy, during the period July 1988 to August 2001. Sustained response was defined as the presence of HCV RNA negativity (determined by using qualitative HCV RNA assay) more than 6 months after the termination of IFN therapy. Diagnosis of HCC was based either on histological examination or on typical computed tomographic and/or angiographic findings at each institution. Patients were excluded if HCC was detected within 1 year after the termination of IFN therapy, because in such cases it was highly likely that the cancer had been present at the end of the IFN therapy. In Japan, at the time of the study, the standard schedule was 6–10 MU IFN-α every day for the first 2–4 weeks and then the same dose given three time a week for the following 20–22 weeks, or 6 MU IFN-β every day for 6–8 weeks.

During the study period at the 16 hospitals, a total of 3504 patients with chronic hepatitis C had received IFN therapy and had been followed up for more than 1 year thereafter, and a sustained response was obtained in 1091 (31.1%) of them. Among the sustained responders, 30 patients (2.7%) developed HCC. By means of the data collection instrument, we requested individual clinical data before IFN therapy for all sustained responders, as well as clinical data at the time of diagnosis of HCC for patients who had developed HCC. The clinical data for all 1091 sustained responders identified were obtained from the 16 hospitals (8 university hospitals and 8 regional hospitals) listed in the appendix. Of these patients, 35 were excluded from the analysis because of the development of HCC within 1 year after IFN therapy (1 patient) or insufficient clinical records before commencement of IFN therapy (34 patients). The final study population comprised a total of 1056 patients showing sustained response to IFN therapy given for chronic hepatitis C, 29 of whom had developed HCC.

Methods

To identify risk factors for the development of HCC in sustained responders to IFN therapy, we used univariate analysis and multivariate analysis to investigate 23 variables before IFN therapy for their relationship to the development of HCC. These variables were chosen by considering possible factors involved in the development of HCC, as indicated by previous investigations,[1–5,10–25] or suggested from our own clinical experience. Each variable, which was classified as host-related or treatment-related, was divided into one of two subgroups on the basis of clinically meaningful values. HCV RNA load was determined quantitatively by competitive reverse-transcription polymerase chain reaction (RT-PCR), branched-DNA probe assay, or Amplicor-HCV monitor assay.[26–28] When the serum HCV RNA level was more than 10^6 equivalents/ml by branched DNA assay, more than 10^6 copies/ml by competitive RT-PCR, or more than 10^5 copies/ml by Amplicor-HCV monitor assay, it was designated as a high viral load; an HCV RNA level of 10^5 copies/ml by the Amplicor-HCV monitor assay has already been demonstrated to correspond to approximately 10^6 equivalents/ml by the branched DNA probe assay or 10^6 copies/ml by competitive RT-PCR.[26–28] HCV subtype was classified by either the method of Okamoto et al.,[29] or Tanaka et al.'s method.[30] Genotypes 1a and 1b corresponded to serological group 1, and genotypes 2a and 2b corresponded to serological group 2, according to the Simmonds et al.[31] classification.[31] The data from liver biopsies that were done within 6 months before IFN therapy were included in this study. Assessments of the staging of liver fibrosis and the grade of inflammatory activity were based on the classification of Desmet and colleagues,[32] in which staging is defined as follows: F0 (no fibrosis), F1 (fibrous portal expansion), F2 (bridging fibrosis), F3 (bridging fibrosis with architectural distortion), and F4 (cirrhosis), and grading is defined as follows: A0 (no activity), A1 (mild activity), A2 (moderate activity), and A3 (severe activity).

To elucidate the clinical features of HCC that developed in sustained responders, 17 variables at the time of diagnosis of HCC were investigated. Number of tumors, maximum tumor size, portal vein invasion, hepatic vein invasion, and bile duct invasion were examined by ultrasonography, computed tomography, and/or angiography. The period to the development of HCC was measured from the day of termination of IFN therapy to the day when HCC was first diagnosed by imaging modalities, such as ultrasonography or computed tomography. The follow-up period for the detection of HCC after termination of IFN therapy was defined as the interval during which checks for HCC were done using tumor markers and/or imaging modalities.

Statistical analysis

Follow up ended with the last recorded visit before August 31, 2001. Incidences were calculated in person-

Table 1. Patient characteristics of 1056 sustained responders to interferon therapy given for chronic hepatitis C

		Number of patients
Host-related variables		
Age (years)	Median (range)	50 (11–76)
Sex	Male	711 (67%)
History of blood transfusion	Positive	266 (27%)
Alcohol abuse[a]	Positive	78 (8%)
Smoking habit[b]	Positive	248 (38%)
HCV viral load	High ($\geq 10^6$)	159 (21%)
HCV serologic group	Group 1	372
	Group 2	466
Hepatitis B surface antigen	Positive	17 (2%)
Treatment-related variables		
Interferon type	α	829 (79%)
	β	166 (16%)
	α + β	61 (6%)
Total amount of interferon (MU)	Median (range)	480 (42–1740)
Treatment period (weeks)	Median (range)	22 (2–56)
Prior interferon therapy	Positive	87

HCV, hepatitis C virus
[a] Alcohol intake, ≥80 g/day × 5 years
[b] Smoking habit, ≥20 cigarettes/day for ≥10 years

years; incidence curves of HCC were calculated by the Kaplan-Meier method; and differences in survival were evaluated by log rank tests. Hazard ratios and trend *P* values were calculated by treating the categories as ordinal variables. The Cox proportional hazard model was used to determine the most significant variables related to the development of HCC. All patients were then assigned a risk index value for the development of HCC, as follows: the value of each factor in the final model was multiplied by its corresponding regression coefficient, and these values were totaled to obtain the risk index for each patient. Stratification of the patients was conducted on the basis of this risk index. All *P* values were two-tailed and were considered significant when less than 0.05.

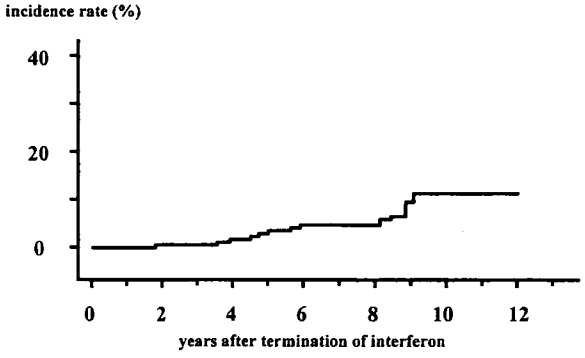

Fig. 1. Cumulative incidence of hepatocellular carcinoma in 1056 sustained responders to interferon therapy given for chronic hepatitis C

Results

Patient characteristics

Table 1 summarizes the patient characteristics of the 1056 sustained responders to IFN therapy given for chronic hepatitis C. The median age was 50 years (range, 11–76) years, and there were 711 men and 345 women (sex ratio, 2.1:1). Hepatitis B surface antigen was positive in 17 patients (2%). The HCV serological group was group 1 in 372 patients and group 2 in 466 patients, and thus a higher proportion of patients were in serological group 2. A total of 829 patients (79%) received IFN-α, 166 patients (16%) received IFN-β, and 61 patients (6%) received both. The median dose and duration of IFN administration were 480 MU and 22 weeks, respectively. No patients received peginterferon or combination therapy with ribavirin, and 87 patients (8%) received more than two cycles of IFN therapy.

Incidence of HCC

Twenty-nine of the 1056 sustained responders developed HCC, with a median follow-up period of 4.7 years. The incidence of HCC per 100 person-years was 0.56 (95% confidence interval, 0.35–0.76), and the incidences of HCC at 3, 5, 7, and 10 years after the termination of IFN therapy were 0.5%, 3.3%, 4.9%, and 11.1%, respectively (Fig. 1).

Univariate analyses

On univariate analysis (Table 2), age more than 60 years, positive smoking habit, platelet count less than $15 \times 10^4/mm^3$, aspartate aminotransferase (AST) more than 100 IU/l, prothrombin time less than 80%, and higher fibrosis stage (incidence of HCC per 100 person-years: F0, 0.00; F1, 0.27; F2, 0.47; F3, 0.62; F4, 1.31) were significant risk factors associated with the development of HCC. Alcohol abuse, total bilirubin, albumin, alanine aminotransferase, virological variables (viral load, serological group), tumor markers (alpha-fetoprotein, protein induced by vitamin K absence or antagonist-II), and treatment-related variables (treatment period, IFN type, total amount of IFN) were not significant risk factors.

Multivariate analyses

All variables whose P values were less than 0.20 on the univariate analyses were entered into the multivariate analyses (Table 3). However, history of blood transfusion, smoking habit, prothrombin time, and indocyanine green retention rate at 15 min (ICG R15) were not included in the model because inadequate data were available. Multivariate regression analysis, which assessed the independent predictive importance of each variable studied for the development of HCC, showed that older age, higher serum AST level, and lower platelet count were significantly related to the development of HCC.

Risk groups based on the regression model

For the clinical application of these findings, a risk index was calculated based on the regression coefficients derived from the three variables identified by multivariate analysis. The index equation was as follows: $1.14 \times$ (0, age $\leq$ 60 years; 1, age $>$ 60 years) + $1.13 \times$ (0, AST $\leq$ 100 IU/l; 1, AST $>$ 100 IU/l) + $1.02 \times$ (0, platelet count $\geq 15 \times 10^4/mm^3$; 1, platelet count $< 15 \times 10^4/mm^3$). The risk index was $\ln[hi(t)/h_0(t)]$, where $hi(t)/h_0(t)$ was the relative risk of the development of HCC for the i-th patient. The index values ranged from 0.00 to 3.29. The patients were then classified into three groups according to the risk index, as follows: low risk, risk index less than 1.00 (equivalent to patients with none of the three risk factors); intermediate risk, risk index from 1.00 to 2.00 (equivalent to patients with one of the three risk factors); and high risk, risk index greater than 2.00 (equivalent to patients with two or more of the three risk factors). The incidence curves for the three groups are shown in Fig. 2. The incidence rates of HCC per 100 person-years (95% confidence interval) in the low-, intermediate-, and high-risk groups were 0.11 (0.00–0.26), 0.44 (0.11–0.77), and 1.98 (1.09–2.87), respectively. There was a significant difference in survival time among the three groups ($P < 0.0001$).

Clinical features of HCC

The characteristics of the 29 patients in whom HCC developed after sustained response are shown in Table 4. All patients were HCV RNA-negative (determined by using qualitative HCV RNA assay), at the time of diagnosis of HCC. Twenty-five patients (86%) were aged 60 years or more, and 24 patients (83%) were men. Among the 13 patients in whom liver biopsy was done at the time of diagnosis of HCC, A0, A1, and A2 histological activity was observed in 5 (38%), 6 (46%), and 2 (15%) patients, respectively. F0, F1, F2, F3, and F4 histological stages were observed in 1 (8%), 1 (8%), 7 (54%), 2 (15%), and 2 (15%) patients, respectively. The median period from the termination of IFN therapy to the development of HCC was 4.6 years (range, 1.4–9.0 years), and there were 11 patients (38%) in whom HCC was detected more than 5 years after the termination of IFN therapy. The periods and methods of medical follow-up examination after the end of IFN therapy varied among the patients, and 8 patients did not receive a sufficient post-treatment medical examination. Among them, HCC of 5 cm or more in size was detected in 5 patients (63%).

Discussion

IFN is already widely used as a standard therapeutic modality for chronic hepatitis C.[5–9] It is generally assumed that eradication of HCV by IFN halts the progression of the disease and prevents clinical complications, including the development of HCC.[5,7,10–14] However, there have been reports of several patients in whom HCC developed after successful IFN therapy.[5,10–25] The incidence and clinical features of HCC, the risk factors for the disease, and the mechanism of carcinogenesis in these patients have not been fully elucidated, because the development of HCC is very rare in sustained responders to IFN therapy. This prompted us to perform a multicenter retrospective cohort study to gather clinical data on such patients.

Of all 1056 sustained responders to IFN therapy in the 16 hospitals in the study, 29 developed HCC, with a median period to development of 4.7 years, and the incidence of HCC was 0.56 (95% confidence interval, 0.35–0.76) per 100 person-years. This value was consistent with the results of previous studies of small numbers of sustained responders to IFN who developed HCC.[5,11–14,20,21–25] This rate was considerably lower than that in IFN-refractory patients or HCV-positive pa-

Table 2. Univariate analysis of 1056 sustained responders in relation to development of HCC

Variables		No. of patients	No. of patients developing HCC	Incidence (95% CI) (/100 person-years)	Hazard ratio (95% CI)	*P* value (log rank)
Host-related variables						
Age	≤60 years	840	13	0.32 (0.14–0.49)	—	
	>60 years	216	16	1.43 (0.73–2.13)	4.23 (2.04–8.80)	0.001
Sex	Male	711	24	0.67 (0.40–0.94)	—	
	Female	345	5	0.30 (0.04–0.57)	0.47 (0.18–1.23)	0.12
History of blood transfusion	Positive	266	11	0.80 (0.33–1.28)	—	
	Negative	723	16	0.45 (0.23–0.67)	0.60 (0.28–1.30)	0.19
Alcohol abuse[a]	Positive	78	2	0.53 (0.00–1.26)	—	
	Negative	946	26	0.56 (0.34–0.77)	1.05 (0.25–4.42)	0.95
Smoking habit[b]	Positive	248	14	1.16 (0.55–1.77)	—	
	Negative	405	7	0.36 (0.09–0.62)	0.30 (0.12–0.75)	0.009
HCV viral load	High (≥10^6)	159	1	0.15 (0.00–0.45)	—	
	Low (<10^6)	593	11	0.42 (0.17–0.66)	2.68 (0.35–20.77)	0.35
HCV serological group	Group 1	372	5	0.27 (0.03–0.52)	—	
	Group 2	466	10	0.47 (0.18–0.76)	1.78 (0.60–5.26)	0.30
Hepatitis B surface antigen	Positive	17	0	0.00		
	Negative	1008	27	0.54 (0.34–0.75)	[c]	0.56
Platelet count (×10^4/mm^3)	≥15	568	7	0.27 (0.07–0.46)	—	
	<15	358	21	1.15 (0.66–1.65)	3.95 (1.68–9.30)	0.002
Total bilirubin (mg/dl)	≥1.0	207	8	0.75 (0.23–1.27)	—	
	<1.0	824	21	0.52 (0.30–0.75)	0.37 (0.32–1.65)	0.45
Albumin (g/dl)	>4.0	564	17	0.59 (0.31–0.87)	—	
	≤4.0	396	8	0.42 (0.13–0.72)	0.78 (0.34–1.80)	0.56
Aspartate aminotransferase (IU/l)	>100	196	13	1.26 (0.57–1.94)	—	
	≤100	844	16	0.39 (0.20–0.58)	0.35 (0.17–0.73)	0.005
Alanine aminotransferase (IU/l)	>100	459	17	0.73 (0.38–1.07)	—	
	≤100	591	12	0.42 (0.18–0.66)	0.63 (0.30–1.32)	0.22
Prothrombin time (%)	≥80	493	9	0.39 (0.14–0.65)	—	
	<80	158	10	1.19 (0.45–1.93)	2.72 (1.10–6.74)	0.03
ICG R15 (%)	≥10	322	9	0.52 (0.18–0.86)	—	
	<10	274	1	0.08 (0.00–0.23)	0.18 (0.02–1.44)	0.11
Alpha-fetoprotein (ng/ml)	>20	66	2	0.58 (0.00–1.39)	—	
	≤20	554	16	0.58 (0.30–0.87)	1.10 (0.25–4.81)	0.78
PIVKA-II (AU/ml)	>0.063	42	0	0.00		
	≤0.063	235	8	0.66 (0.20–1.12)		0.63
Histological activity grade	A0 (No)	12	0	0.00		
	A1 (Mild)	309	6	0.40 (0.08–0.73)		
	A2 (Moderate)	359	11	0.64 (0.26–1.01)		
	A3 (Severe)	169	5	0.61 (0.07–1.14)	1.28 (0.74–2.21)	0.39
Histological fibrosis stage	F0 (No)	26	0	0.00		
	F1 (Mild)	405	5	0.27 (0.03–0.50)		
	F2 (Moderate)	301	7	0.47 (0.12–0.82)		
	F3 (Severe)	170	6	0.62 (0.12–1.11)		
	F4 (Cirrhosis)	97	4	1.31 (0.03–2.60)	1.56 (1.03–2.36)	0.03
Treatment-related variables						
Treatment period (weeks)	≥24	472	17	0.73 (0.38–1.08)		
	<24	584	12	0.41 (0.18–0.65)	0.56 (0.27–1.16)	0.11
Interferon type	α	829	25	0.61 (0.37–0.85)	—	
	β	166	4	0.55 (0.01–1.10)	0.99 (0.34–2.86)	0.98
	α + β	61	0	0.00	[c]	
Total amount of interferon (MU)	>500	491	10	0.42 (0.16–0.68)	—	
	≤500	534	16	0.60 (0.31–0.89)	1.34 (0.61–2.95)	0.47
Prior interferon therapy	Positive	87	2	0.46 (0.00–1.10)	—	
	Negative	955	27	0.57 (0.36–0.79)	1.17 (0.28–5.00)	0.82

HCC, hepatocellular carcinoma; CI, confidence interval; HCV, hepatitis C virus; ICG R15, indocyanine green retention rate at 15 min; PIVKA II, protein induced by vitamin K absence or antagonist-II; —; reference category

[a] Alcohol intake ≥80 g/day + 5 years

[b] Smoking habit, ≥20 cigarettes/day for ≥10 years

[c] not estimated

tients who did not receive IFN therapy, which has been reported to be 1.4%–7% yearly,[4–7,10–13,21–24] and it was obvious that IFN therapy decreased the risk of HCC in sustained responders. However, the incidence of HCC gradually increased over a period of at least 9 years after the termination of IFN therapy (Fig. 1). This suggests that the risk of HCC is not completely eliminated in patients who have a sustained response to IFN therapy,

at least for up to 9 years following cessation of the treatment.

Identification of the risk factors for the development of HCC in sustained responders is important, so that high-risk patients can be screened carefully for early detection of HCC and given potentially curative treatments such as hepatic resection; such patients generally have a good hepatic reserve after the elimination of HCV. Among the variables we investigated, multivariate analysis showed age to be an independent risk factor. As the patient ages, the period of HCV infection becomes longer, and the liver becomes more severely cirrhotic. Therefore, advanced age may simply represent the progression of associated liver disease. These findings are compatible with previous reports of the development of HCC in patients with chronic hepatitis C.[11-14,20-22]

Serum AST level and platelet counts were also independent risk factors in the present study. Some studies have reported that increased AST level and decreased platelet count are correlated with the progression of liver fibrosis,[33-34] which has been reported to be one of the most important risk factors for the development of HCC in patients with chronic hepatitis C.[5,11-13,21] Progression of liver fibrosis may reduce the clearance of AST,[35] leading to increased serum AST levels.[36] This progression is also associated with decreased production of thrombopoietin by hepatocytes[37] and progressive hypersplenism with worsening portal hypertension;[38] and, hence, reduced platelet production and increased platelet destruction. Moreover, in the present study, these factors were strongly associated with histological stage (Pearson's correlation coefficient; $P < 0.0001$). Therefore, increased AST level and decreased platelet count may reflect more progressive liver fibrosis.

For the clinical application of these findings, we proposed a risk index based on the independent risk factors. Patients were classified into three groups, with low, intermediate, and high risk ($P < 0.0001$ for difference in survival time among the three groups; Fig. 2). This index can be easily calculated, because it is based on variables obtained during routine laboratory examinations before IFN therapy is begun. This index, therefore, may be helpful in assessing the risk of development of HCC after sustained response to IFN therapy, although it is also important to validate this risk index by applying it to other populations of patients. Patients in the high-risk group (incidence rate, 1.98 per 100 person-years) may benefit from regular diagnostic imaging for the early detection of HCC.

In the analysis of the clinical features of HCC there were no specific findings. The period to the development of HCC after IFN therapy (median, 4.6 years; 1.4–9.0 range, years) was variable. HCC developed even in two patients whose liver showed improvement to mild fibrosis (stage F0 or F1) and in five patients whose liver improved to no activity (A0) after IFN therapy. The follow-up periods and methods for the detection of HCC after the termination of IFN therapy varied among the patients, and in some patients HCC was detected at far more advanced stages than in others, because of insufficient follow up after IFN therapy. This finding may suggest the need for regular follow up by diagnostic imaging, even after sustained response to IFN therapy for chronic hepatitis C, especially in the high-risk group.

Our study involved some uncertainties. First, because the study was retrospective, many data items were missing from the replies to the data collection instrument, and we had to ignore unmeasured or unrecorded data when conducting the statistical analyses. In the multivariate analysis, therefore, only variables whose P values were less than 0.20 on the univariate analysis were entered. Also, history of blood transfusion, smoking habit, prothrombin time, and ICG R15, whose P values were lower than 0.20, had to be excluded from the model because of missing data; these factors were potentially significant on multivariate analysis. Secondly, we sought information on serum hepatitis B virus DNA

Table 3. Significant risk factors identified in 1056 sustained responders, as determined by multivariate analysis with the Cox proportional hazard model

Variable	Hazard ratio (95% confidence interval)	P value
Age	3.13 (1.32–7.42)	0.01
Aspartate aminotransferase	3.10 (1.31–7.31)	0.01
Platelet count	2.78 (1.07–7.20)	0.04

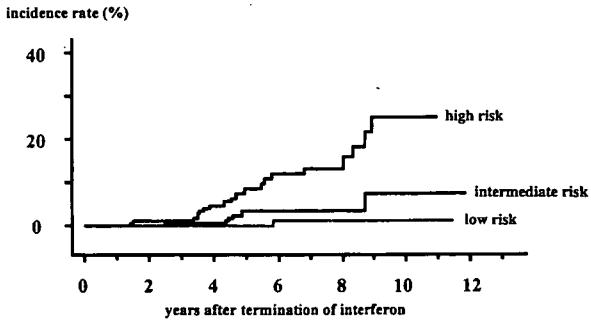

Fig. 2. Cumulative incidence of hepatocellular carcinoma for the three groups determined by a risk index based on the results of multivariate analysis. Low risk (risk index < 1.00); intermediate risk (risk index from 1.10 to 2.00); high risk (risk index, ≥2.00)

Table 4. Clinical features at the time of diagnosis of HCC in 29 patients who developed hepatocellular carcinoma after sustained response to interferon therapy given for chronic hepatitis C

Age (years)	Sex	HCV RNA	HBs Ag	Histological fibrosis stage	Histological activity grade	AFP (ng/ml)	PIVKA II (AU/ml)	Number of tumors
64	Male	Negative	Negative	NA	NA	2	0	4
60	Male	Negative	Negative	NA	NA	51	0.211	1
38	Male	Negative	Negative	NA	NA	4.3	NA	>5
67	Male	Negative	Negative	F4	A0	4.2	0.033	1
75	Male	Negative	Negative	F1	A1	5	0.054	1
65	Female	Negative	Negative	NA	NA	5	0.029	1
62	Male	Negative	Negative	NA	NA	3	NA	1
61	Male	Negative	Negative	F2	A0	3	0.001	1
64	Male	Negative	Negative	F2	A0	4	0.426	1
70	Male	Negative	Negative	NA	NA	46 000	NA	1
64	Male	Negative	Negative	NA	NA	146	0.049	1
54	Female	Negative	Negative	F3	A1	2165	6690	1
65	Male	Negative	Negative	F4	A2	25.9	0.015	>5
61	Male	Negative	Negative	F2	A1	4	1.79	1
64	Male	Negative	Negative	F2	A0	NA	NA	1
63	Male	Negative	Negative	NA	NA	135.3	0.06	1
67	Male	Negative	Negative	NA	NA	3.5	0.013	1
75	Male	Negative	Negative	NA	NA	2	NA	1
62	Male	Negative	Negative	F2	A1	1026	13.32	1
62	Male	Negative	Negative	F2	A1	2.3	1.79	1
68	Female	Negative	Negative	F3	A2	9.1	0.016	1
59	Male	Negative	Negative	F0	A0	29	0.029	1
70	Male	Negative	Negative	NA	NA	488.3	601 371	1
54	Male	Negative	Negative	NA	NA	258	2.1	1
68	Female	Negative	Negative	NA	NA	2.8	0.023	1
60	Male	Negative	Negative	F2	A1	3.2	0.023	1
70	Male	Negative	Negative	NA	NA	5463	6.566	2
70	Female	Negative	Negative	NA	NA	464.2	NA	1
77	Male	Negative	Negative	NA	NA	72	0.136	2

NA, not available; HBs Ag, hepatitis B surface antigen; AFP, alpha-fetoprotein; PIVKA II, protein induced by vitamin K absence or antagonist-II; Vp, portal vein invasion; Vv, hepatic vein invasion; B, bile duct invasion; US, ultrasonography; CT, computed tomography

in sustained responders in whom HCC developed after successful IFN therapy, but data could be obtained for only two patients, who were negative for hepatitis B virus DNA. We cannot rule out the presence of occult hepatitis B virus in the other patients, although all patients were negative for hepatitis B antigen. In spite of these uncertainties, this study represents a comprehensive analysis of HCC developing after sustained response to IFN therapy, because we were able to collect clinical data for a large number of sustained responders at 16 major hospitals.

In this study, we encountered 29 patients in whom HCC developed after successful IFN therapy, but the reason why HCC developed in these sustained responders is unclear. The existence of a small undetected HCC at the time of IFN therapy may have been responsible for the appearance of HCC after the sustained response to IFN therapy. However, in 11 patients (38%), HCC was detected more than 5 years after IFN therapy, and the incidence of HCC gradually increased for at least 9 years after IFN therapy. Considering the late onset of HCC in these patients, we cannot neglect the possibility of the de-novo development of HCC after the eradication of HCV. HCV is a single-stranded RNA virus without a DNA intermediate in its replicative cycle, so that the integration of HCV nucleic acid sequences into the host genome seems unlikely. Therefore, it is difficult to believe that HCV itself is a causative factor of HCC in the absence of chronic inflammation, liver cell necrosis and regeneration, and extensive fibrosis. It is probable that carcinogenesis is not a single-step event, but a complex multistep process. Future studies should aim to define the basic oncogenic mechanisms by which sustained responders to IFN develop HCC. Exploration of these mechanisms may point the way toward new strategies for the prevention of HCC.

In conclusion, some patients showing a sustained response to IFN therapy given for chronic hepatitis C demonstrated potential for the development of HCC for up to 9 years following cessation of the treatment. This suggests that the risk of HCC in sustained responders is not completely eliminated. The establishment of risk factors and an index for the development of HCC may be useful in determining follow-up strategy in patients after a sustained response to IFN therapy given for chronic hepatitis.

Table 4. *Continued*

Maximum tumor size (mm)	Vp	Vv	B	Differentiation of HCC	Period to development Of HCC (years)	Medical follow-up period (months)	Diagnostic modality
18	0	0	0	Moderately	1.43	3	US
16	0	0	0	NA	1.51	1	US
>20	3	0	0	NA	1.79	None	US
15	0	0	0	Moderately	2.52	1	US
25	0	0	0	Moderately	3.32	2	CT
20	0	0	0	Well	3.39	3	US
34	2	1	2	Well	3.54	2	US
20	0	0	0	Well	3.59	3	Laparoscopy
40	0	0	0	NA	3.70	None	US
50	2	2	2	NA	3.89	None	US
30	0	0	0	Well	4.35	1	US
110	0	0	0	Poorly	4.38	6	US
15	0	0	0	Well	4.48	6	US
50	1	0	1	Moderately	4.58	12	US
80	0	0	0	Moderately	4.60	None	CT
NA	0	0	0	NA	4.70	6	US
44	0	0	0	NA	4.88	6	US
28	0	0	0	NA	4.97	3	US
60	1	1	1	Moderately	5.52	None	US
50	1	0	1	Moderately	5.58	6	US
51	0	0	0	Combined type	5.80	3	US
40	0	0	0	Moderately	5.86	None	US
>20	2	0	0	NA	6.61	3	US
150	3	0	0	Poorly	6.86	None	US
15	0	0	0	NA	8.05	3	US
15	0	0	0	Well	8.39	6	US
60	0	0	0	Well	8.78	None	US
16	0	0	0	NA	8.79	3	US
42	0	0	0	NA	8.98	1	CT

Appendix

In addition to the study authors' hospitals (the four institutions listed on the title page), data were supplied by the following hospitals and clinics in the Kyushu Division of the Japanese Society of Gastroenterology: Shinnittetsu Yahata Memorial Hospital; Yame General Hospital; First Department of Internal Medicine, Ryukyu University School of Medicine; Second Department of Internal Medicine, Kagoshima University School of Medicine; Hayato Town Medical Association Medical Center; Department of Internal Medicine, Saga Medical School; Department of Medicine and Biosystemic Science, Kyushu University School of Medicine; Nishinihon Hospital; Kagoshima Kouseiren Hospital; Miyata Memorial Hospital; Second Department of Internal Medicine, Nagasaki University School of Medicine; and Yonabaru Central Hospital.

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EVALUATION OF A HEPATITIS B VACCINATION PROGRAM IN CHIANG MAI, THAILAND

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Abstract. Chiang Mai is a province in northern Thailand that started a vaccination program for hepatitis B virus (HBV) infection in 1989. In this paper, we report the long-term efficacy of this program. Of children aged 4-9 years, 65.7% had a complete course and 3.8% had an incomplete vaccination course. Urban schoolchildren had higher percentage of HB vaccination than rural schoolchildren (89.1% *vs* 46.9% for the complete course, $p < 0.001$). The overall prevalence rate of HBsAg in Chiang Mai schoolchildren was 1.2%, with no significant differences between gender ($p = 0.496$) and school areas ($p = 0.477$). Anti-HBc antibodies were detected in 6.9% of children. Overall, 26.2% of children had protective levels of anti-HBs antibodies (≥10.0 mIU/ml), and 11.2% had low levels of these antibodies (1.0-9.9 mIU/ml). Compared to previous reports, our results show a lower percentage of anti-HBs antibodies, 33.8% of children age 4 years had protective anti-HBs antibodies, dropping to 18.4% by age 9 years. Among those anti-HBs seropositive, 9.1% were anti-HBc positive, indicating a natural infection with HBV. We found a small number of children, despite adequate immunization, developed HBV infection.

INTRODUCTION

Hepatitis B virus (HBV) infection is endemic in Southeast Asia and Africa, and is transmitted by parenteral routes, maternal-infant exposure, and horizontal spread between children (Merican *et al*, 2000). Almost all HBV-infected infants become carriers of HBV, and act as sources of the infection to their family and community. Such individuals are at significant risk for developing chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Thus, control of HBV infection and interruption of its spread are important public health goals. The most important method of prevention of HBV infection is the vaccination of newborns and children with hepatitis B (HB) vaccine. Studies showed that before immunization, about 20% of children under 5 had serologic evidence of HBV infection, rising to 70% by age 15 (Grossman *et al*, 1975). About 4% of non-immunized Thai children were carriers of hepatitis B (Luksamijarulkul *et al*, 1995), reaching 8% by adulthood (Grossman *et al*, 1975; Merican *et al*, 2000). Population-based studies have shown that the use of the HB vaccine in infants can reduce the HBV chronic carrier prevalence from high (>8%) to low (<2%) in immunized cohorts of children (Kane, 1998).

Thailand has undertaken a systematic approach toward control of HBV infection. Before systematic immunization, one study found that 5.7% of children acquired the infection in a one-year period (Kozik *et al*, 2000). The carrier rate in Thai children age 2-16 years was found to be 13% of those who were infected with HBV (Kozik *et al*, 2000). In 1989, the Thailand Ministry of Public Health (MOPH) established a pilot project of HB immunization in Chiang Mai and Chon Buri Provinces demonstrating that HB vaccine can be effectively administered along with other Expanded Program of Immunization (EPI) vaccines. In 1992, the Thai Government integrated the HB vaccine into the national EPI. Children receive 0.5 ml of HB vaccine intramuscularly within 7 days of birth, at 2 months and at 6 months of age. The HB vaccines given to older children varied with the year of inoculation. In 1989, a

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plasma-derived vaccine (the Cheil Suger, Korea), with a 3 μg per dose, was used. From 1990 to 1994, the plasma-derived vaccine produced by Korean Green Cross Corporation (Korea) was administered at 10 μg per dose. Since 1995, the 10 μg per dose of recombinant Engerix B® vaccine (SmithKline Biologicals, Belgium) has been used (Poovorawan *et al*, 2001). Long-term evaluation of this project in Chiang Mai, where the project began, has not been done. In this paper, we report the results of hepatitis B vaccination in Chiang Mai, Thailand, and estimate the efficacy of this program.

MATERIALS AND METHODS

Study population

From July 1998 to August 2000, children aged 4-9 years were randomly selected from 7 rural schools (377 children, including 185 males and 192 females) and 3 urban schools (303 children, including 147 males and 156 females) in Chiang Mai Province, northern Thailand. The purpose of the study was discussed with the parents or guardians, and written consent was obtained in all cases. A questionnaire was completed and personal vaccination records were reviewed. A total of 680 blood samples were obtained from the schoolchildren (332 males and 348 females). Serum samples were prepared on the day they were obtained, stored at 4°C for not more than 3 days, or stored at -20°C until tested.

Serologic studies

A qualified technician tested for the presence of HBsAg using the Monolisa Ag HBs second generation ELISA kit (Sanofi Diagnostic Pasteur, Manes la Coquette, France). Testing for anti-HBs antibodies was done using the Monolisa Anti-HBs 3.0 kit together with the 5 points calibration Monolisa Anti-HBs Standard (negative control, and 10, 50, 100, and 150 mIU/ml) (Sanofi Diagnostic Pasteur). All positive samples were retested, and all remained positive. The anti-HBs antibody titers were reported as the average value between the initial positive test and the repeated test. Levels above 10.0 mIU/ml were considered to be protective; anti-HBs and levels between 1.0-9.9 mIU/ml were considered to be low.

Data analysis

Data were analysed by determining the percentages of hepatitis B vaccine coverage and each viral marker obtained per population group. Prevalence among the different groups was compared using χ^2 or Fisher's exact test as appropriate. Results were considered statistically significant when $p < 0.05$.

RESULTS

Coverage of the HB vaccination program

Children aged 4-9 years should reflect the status of subjects born after the HB immunization program in Chiang Mai started in 1989.

Older age schoolchildren represented children at the start of the program. Younger age schoolchildren represent children under the current national EPI program. The children were divided into three groups: those who received a complete course of HB vaccination (*ie*, 3-5 doses of HB vaccine); those who received an incomplete course of HB vaccination (1-2 doses of HB vaccine) and those who did not received HB vaccination. This information was obtained by questionnaire along with review of the personal vaccination records. The results by age group and children school areas are presented in Fig 1. Of the 680 children, 447 (65.7%) had a history of complete HB vaccination, including 221 males (66.6%) and 226 females (64.9%); 26 (3.8%) had

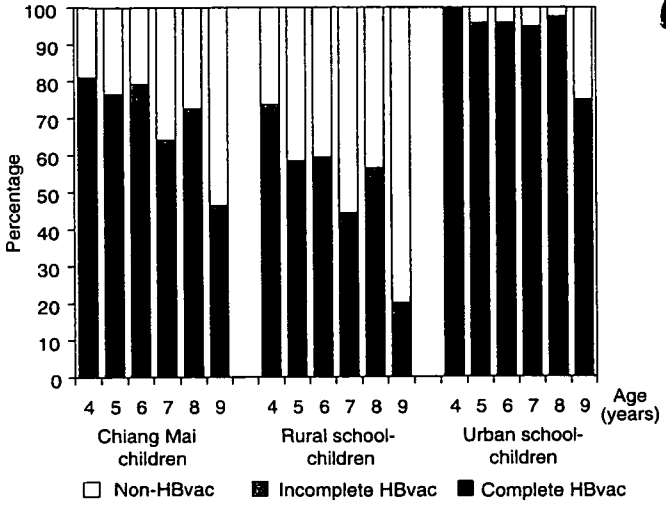

Fig 1–Coverage of hepatitis B vaccination in Chiang Mai children.

Table 1
HBsAg and anti-HBc positivity among Chiang Mai children by age and comparison between school areas.

Age (years)	HBsAg positive (%)			Anti-HBc positive (%)		
	Chiang Mai children	Rural school-children	Urban school-children	Chiang Mai children	Rural school-children	Urban school-children
4-5	1/223 (0.4%)	0/130 (0.0%)	1/93 (1.1%)	18/223 (8.1%)	16/130 (12.3%)	2/93 (2.2%)
6-7	2/230 (0.9%)	1/120 (0.8%)	1/110 (0.9%)	13/230 (5.7%)	5/120 (4.2%)	8/110 (7.3%)
8-9	5/227 (2.2%)	2/127 (1.6%)	3/100 (3.0%)	16/227 (7.0%)	10/127 (7.9%)	6/100 (6.0%)
Total	8/680 (1.2%)	3/377 (0.8%)	5/303 (1.7%)	47/680 (6.9%)	31/377 (8.2%)	16/303 (5.3%)

a history of incomplete HB vaccination, including 14 males (4.2%) and 12 females (3.4%). Children in urban schools showed a much higher prevalence in terms of HB vaccination than children in rural schools (89.1% *vs* 46.9% for a complete course, $p < 0.001$). This difference was not related to the sex of the children, $p = 0.722$. In urban schools, 87.8% of males and 90.4% of females received a complete course, and in rural schools, 49.7% of males and 44.3% of females did. The coverage of HB vaccination increased over time. For the oldest age group (9 years), 49/125 (39.2%) and the youngest age group (4 years), 59/74 (79.7%) had received a complete course of vaccination. Most of these children were from urban schools, 68.3% of the oldest age group (9 years) and 100% in the youngest age group (4 years) had undergone a complete course of vaccination, compared to 12.3% in the oldest age group and 71.7% in the youngest age group of children from rural schools.

Prevalence of HBV infection in Chiang Mai children

The results of testing for HBsAg and anti-HBc are presented in Table 1. The average prevalence rate of HBsAg in Chiang Mai schoolchildren age 4-9 years was 1.2% (8/680). There was a general increase in the prevalence rate with age, reaching 2.2% in the 8-9 years age group. The positive rate in males was 1.5% (5/332) and in females was 0.9% (3/348), which was not statistically different ($p = 0.496$). HBsAg positive rates in urban schoolchildren (1.7%) and rural schoolchildren (0.8%) were not significantly different, $p = 0.477$.

Another indicator of true infection by HBV is the development of anti-HBc antibodies. The overall prevalence of anti-HBc antibodies was 6.9% (47/680), with no significant difference between the sexes (6.9% in males and females, $p = 0.999$). When rural and urban children were considered separately, the prevalence in the rural children (8.2%) was not significantly different from urban children (5.3%), $p = 0.133$.

Surprisingly, all 5 HBsAg positive children from urban schools had a history of HB vaccination (two at 9 years of age, one at 7 years of age and one at 4 years of age had complete HB vaccination and one at 9 years of age had an incomplete HB vaccination). Most of them were anti-HBc positive, except the one 9 years of age who was anti-HBc negative (who had a history of complete HB vaccination). All 3 HBsAg positive children from rural schools were non-vaccinated and anti-HBc positive, two at 9 years of age and one at 6 years of age.

Efficacy of the vaccination program

A simple indicator of efficacy is the seroprevalence of anti-HBs antibodies. These values are presented in Fig 2. In our study group, we found that of those age 4-9 years, 178 (26.2%) had protective anti-HBs and 76 (11.2%)

Table 2
Anti-HBc positivity among Chiang Mai children with anti-HBs negative and positive by age groups and comparison between school areas.

Age (years)	Anti-HBc positive/Anti-HBs negative			Anti-HBc positive/Anti-HBs positive		
	Chiang Mai children	Rural school-children	Urban school-children	Chiang Mai children	Rural school-children	Urban school-children
4-5	8/119 (6.7%)	7/73 (9.6%)	1/46 (2.2%)	10/104 (9.6%)	9/57 (15.8%)	1/47 (2.1%)
6-7	5/152 (3.3%)	1/82 (1.2%)	4/70 (5.7%)	8/78 (10.3%)	4/38 (10.5%)	4/40 (10.0%)
8-9	11/155 (7.1%)	7/93 (7.5%)	4/62 (6.5%)	5/72 (6.9%	3/34 (8.8%)	2/38 (5.3%)
Total	24/426 (5.6%)	15/248 (6.0%)	9/178 (5.1%)	23/254 (9.1%)	16/129 (12.4%)	7/125 (5.6%)

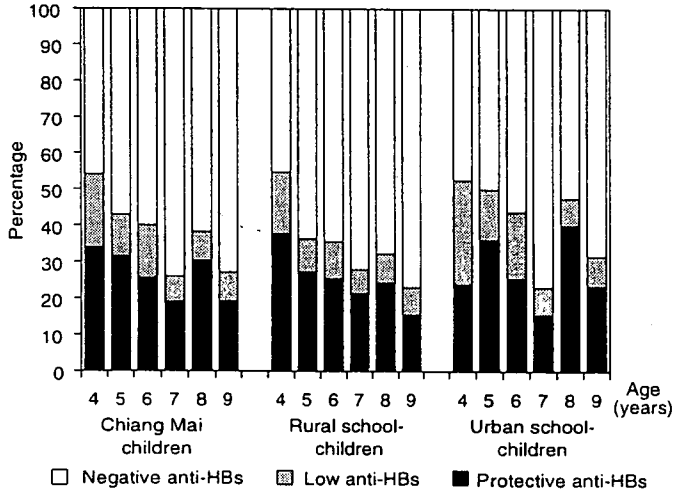

Fig 2–Seroprevalence of anti-HBs levels in Chiang Mai schoolchildren, by age and areas (rural *vs* urban).

had low levels of anti-HBs. The prevalence decreased with age, from 33.8% with protective anti-HBs and 20.3% with low levels of anti-HBs at age 4 years to 18.4% with protective anti-HBs and 8.0% with low levels of anti-HBs by age 9 years. No significant difference was observed between male and female children, p = 0.096. When separated into rural and urban groups, the seroprevalence of anti-HBs antibodies in urban children (28.1% with protective anti-HBs) was not significantly different from rural schoolchildren (24.7% with protective anti-HBs), p = 0.131.

Efficacy may be more accurately judged by the development of protective levels of anti-HBs antibodies (≥10 mIU/ml). Compared to the coverage with HB vaccination, the proportion of protective anti-HBs antibodies is quite low, demonstrated by Figs 1 and 2. The coverage of complete HB vaccination was 65.7%. The presence of protective anti-HBs in our study group was 26.2%. In the youngest age group (4 years) with 79.7% with complete HB vaccination, 33.8% had protective levels of antibodies. Even in the youngest age group (4 years) of urban schoolchildren with 100% complete HB vaccination, 23.8% had protective levels of antibodies.

The presence of anti-HBc antibodies indicates natural infection with HBV. The prevalence of anti-HBc in anti-HBs seronegative and anti-HBs seropositive individuals is presented in Table 2. The prevalence of anti-HBc among the anti-HBs seropositive group (9.1%) was not significantly different from the anti-HBs seronegative group (5.6%), p = 0.089. In the anti-HBs seropositive group, the prevalence of anti-HBc in rural schoolchildren was 12.4%, while in the urban schoolchildren it was 5.6%, which was not significantly different (p = 0.059).

DISCUSSION

From 1989 to 1992, the Thailand's HB immunization model program was conducted in Chiang Mai and Chon Buri Provinces and demonstrated that HB vaccine could be effectively administered along with other EPI vaccines. By the end of the project, overall coverage for complete HB immunization in Chiang Mai had

reached 93.1%. A minority of children received incomplete HB immunization or no immunization. HB vaccination was shown to provide over 80% protective efficacy against HBV infection (Chunsuttiwat *et al*, 1997). Stepwise expansion of HB vaccination evolved into a nationwide program in 1992. The HB vaccination coverage rate has been rapidly catching up with its EPI counterparts, with the coverage rate of the third dose ranging from 71.2-94.3% (Chongsrisawat *et al*, 2000; Poovorawan *et al*, 2001). We found that the coverage rates of Chiang Mai children age 4-9 years old, who should have received 3 doses of HB vaccine, was 65.7%. Some children received incomplete HB immunization (3.8%). In Chon Buri, after its integration into the EPI program, the complete HB vaccination rate was 71.2% and the incomplete HB vaccination rate was 12.9% (Poovorawan *et al*, 2001). There was a marked difference between the urban and rural environments. City children had a coverage rate of 89.1% compared to only 46.9% for rural children. These results are slightly less than those reported in other study. These numbers may be an underestimate, since the data were obtained by questionnaire and from health record booklets, and some of which had been lost, especially in older children and in rural schools.

The prevalence of HBsAg can be used as an indicator of true HBV infection, as opposed to anti-HBs antibodies, which develop following infection and immunization. Other studies have shown that the prevalence of HBsAg in non-immunized children is 3.64% (Luksamijarulkul *et al*, 1995), but this figure has dropped by 85% after the immunization program was instituted (Chongsrisawat *et al*, 2000), with a prevalence of 0.67% reported (Poovorawan *et al*, 2000). The current carrier rate in Thai children has been determined to be 0.55-7% (Chub-uppakarn *et al*, 1998; Poovorawan *et al*, 2000; 2001). In Chon Buri, after the integration of the HB vaccine into the EPI, the HBsAg positive rate was 0.7% (Poovorawan *et al*, 2001). In Chiang Mai, 1.2% of all children had circulating HBsAg in serum, a figure comparable to other studies. The presence of anti-HBc antibodies can also serve as an indicator of infection by HBV. Other studies have found that 5.5% of children 1-10 years old have anti-HBc antibodies (Poovorawan *et al*, 2000), compared to 6% of non-immunized children (Luksamijarulkul *et al*, 1995). In our study group, we found the prevalence rate among children 4-9 years old was 6.9%. While in Chon Buri, after the integration of HB vaccine into the EPI, the anti-HBc positive rate was 6.3% (Poovorawan *et al*, 2001).

The efficacy of immunization can be evaluated by measuring the levels of protective anti-HBs antibodies. By age 10, about 56% of immunized children have antibodies to HBsAg (Poovorawan *et al*, 2000), compared to 15% of non-immunized children (Luksamijarulkul *et al*, 1995). The prevalence rate is higher in younger children, with values of 94% at 0-2 years of age, dropping to 76% by 3-5 years of age (Chub-uppakarn *et al*, 1998). In our study, 33.8% of children 4 years old had protective anti-HBs antibodies, dropping to 18.4% by age 9 years. The prevalence of samples with anti-HBs antibodies declined as children got older, except for an increase in the 8-year old age group (30.4%). The pattern of anti-HBs present is similar to the pattern of the coverage of HB vaccination, by age and area (rural *vs* urban). Compared to previous reports, our results show a lower percentage of anti-HBs antibodies. Anti-HBs antibodies may be from natural infection with HBV. This was demonstrated by the fact that 9.1% of children with anti-HBs were anti-HBc positive.

In our study group, there were some apparently completely immunized children who had evidence of true HBV infection with circulating HBsAg and/or anti-HBc antibodies. Children in Thailand are not screened for previous HBV infection prior to immunization. The children with anti-HBc antibodies may well have acquired the natural infection before they were immunized. The three HBsAg positive children from rural schools were non-vaccinated and had anti-HBc as a result of natural infection. While the five HBsAg positive children from urban schools had a history of HB vaccination (four had complete HB vaccination and one had incomplete HB vaccination). Most of them were anti-HBc positive, except one who was anti-HBc negative (had a history of complete HB vaccination). The children with circulating HBsAg despite immuniza-

tion were true failures of vaccination. These children have the potential to become chronic carriers of HBV. These results imply failures or gaps in the immunization program. Our study did not uncover any specific source of the problem. Several factors, such as the commercial source of the vaccines, inadequate transportation and storage systems, and inefficient methods of administration might reduce the effectiveness of the vaccine. More recently, new variants of HBV have been reported that occur more frequently in vaccinated individuals (Theamboonlers *et al*, 2001). These viruses have critical amino acid differences that allow them to escape the host immune system and the protective effects of the vaccine.

Previous studies have reported seroprevalence based on any positive value for anti-HBs antibodies. In our study, we distinguished between positive serology for anti-HBs antibodies as protective levels (≥10.0 mIU/ml) and low levels (1.0-9.9 mIU/ml) ≥ of these antibodies. We found that, overall 26.2% of children had protective levels of anti-HBs antibodies, and 11.2% had low levels of these antibodies. After vaccination, the strongest antibody response was detected within the first year, and after approximately 5 years it decreased to low or undetectable levels in some individuals. Most studies in Thailand have suggested that a booster dose after the initial three doses is not necessary, and that immunologic memory provides adequate protection, even if levels of anti-HBs antibodies are below 'protective' levels (Chongsrisawat *et al*, 2000; Poovorawan *et al*, 2000). The presence of HBsAg/anti-HBc in vaccinated children from urban schools with high coverage under the HB vaccination program call for evaluation HBV infection after HB vaccination. Some children can become chronic carriers, despite adequate vaccination.

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