patients from surviving patients was set between 4 and 5. Consequently, excellent accuracies were obtained through analysis in patients belonging to the estimation cohort; predictive accuracy was 0.78 with either of PPV, NPV, sensitivity and specificity greater than 0.7. Such high predictive accuracy was also found through analysis in patients belonging to the validation cohort. It is noteworthy that peripheral platelet count and the presence of liver atrophy were added to the list of predictive variables in the present scoring system. Also, cut-off values to grade other variables, such as the interval between the onset of hepatitis symptoms and the development of hepatic encephalopathy, differ between the present system (Table 6) and previous guidelines (Table 1). These modifications may contribute to improve predictive accuracy of the novel scoring system when applied to recent patients.

In the present study, the age of patients was excluded from the list of predictive variables to facilitate the use of the system in pediatric patients. In our database, a patient showing total score of 6 died, while three cases with total scores of 4 or less survived, when the system was applied for patients aged less than 15 years old. Furthermore, the most recent report by Fujisawa showed 100% specificity and PPV by the scoring system in 40 pediatric patients.[12] Thus, the system seems to be useful even in such patients. Also, plasma HGF concentration was deleted from the list of predictive variables, because it is difficult to obtain the results within a day in most of the hospitals in Japan. In contrast, the presence of liver atrophy was included in the predictive variable list, but the quantitative criteria for liver atrophy were not specified in the present scoring system. The estimated liver volume is measured on CT examination, and the ratio of the value to the standardized liver volume was reported to correlate with mortality in patients with acute liver failure in Japan.[13] These problems, regarding age of patients, significance of plasma HGF concentration and diagnostic criteria to determine liver atrophy should be further investigated.

In conclusion, a novel scoring system for predicting outcome of patients with fulminant hepatitis and LOHF was established. This system may be useful to determine the indication of liver transplantation in patients with acute liver failure, since the system showed high predictive accuracies even after the validation.

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REVIEW

Changing etiologies and outcomes of acute liver failure: A perspective from Japan

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Key words
acute liver failure, fulminant hepatitis, Japan, liver transplantation, viral hepatitis.

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Abstract

Acute liver failure in Japan usually consists of fulminant hepatitis (FH) due to viral infection, autoimmune hepatitis and drug-allergy-induced liver injury. The annual incidence of FH was estimated at 429 cases in 2004. FH is classified into acute or subacute type, and the prognosis of the latter is poor. Hepatitis B virus (HBV) is the most frequently identifiable agent that causes FH in Japan. Transient HBV infection is more prevalent in the acute than subacute type, whereas the frequency of HBV carriers is greater in the subacute type. FH due to HBV reactivation from resolved hepatitis B has been increasingly observed in patients with malignant lymphoma treated with rituximab and corticosteroid combination therapy. The prognosis is poor in HBV carriers with acute exacerbation, especially in patients with HBV reactivation from resolved hepatitis B. Despite careful investigation, the etiology is still unknown in 16% and 39% of the acute and subacute type of FH, respectively. Autoimmune hepatitis and drug-allergy-induced liver injury are found in 7% and 10%, respectively, and are more frequently observed in the subacute type of FH. Living donor liver transplantation is now the standard care for individuals with poor prognosis. Artificial liver support with plasmapheresis and hemodiafiltration plays a central role while waiting for a donor liver or for the native liver to regenerate. Further research is necessary to identify the causes of unknown origin. In addition, to improve the prognosis of FH, it is necessary to establish treatment modalities that are effective for liver regeneration.

Introduction

Acute liver failure is a clinical syndrome that is marked by the sudden loss of hepatic function in a person without chronic liver disease. The causes of acute hepatic failure are varied and differ geographically. In Japan, fulminant hepatitis (FH) is defined as having hepatitis, when grade II or worse hepatic encephalopathy develops within 8 weeks of the onset of the disease symptoms, with a prothrombin time of ≤40%. FH due to viral infection, autoimmune hepatitis and drug-allergy-induced liver injury is the main cause of acute liver failure in Japan. In contrast, other causes, including paracetamol overdose, other drug toxicity, metabolic liver disease, and acute fatty liver of pregnancy, are infrequent.

The Intractable Hepato-biliary Diseases Study Group of Japan annually performs a nationwide survey of patients with FH and late-onset hepatic failure (LOHF). This paper summarizes the results of the survey and addresses the characteristics and trends of acute liver failure in Japan.

Definition and methods

In 1969, Trey and Davidson defined acute liver failure as the occurrence of encephalopathy within 8 weeks of the onset of acute hepatic illness, and in the absence of pre-existing liver disease.[1] Thereafter, patients with hepatic encephalopathy that develops between 8 and 24 weeks after disease onset are defined as having LOHF.[2] Other definitions based on the duration of illness have subsequently been used to classify patients:[2–4] hyperacute, <7 days; acute, 7–28 days; and subacute, 28 days to 6 months. In Japan, patients with FH are classified into acute or subacute type, in which the encephalopathy occurs within 10 days, or later than 11 days, respectively, of the onset of disease symptoms.[5,6] Based on the previous survey, patients with FH who present within 10 days of symptom onset have significantly higher survival rates than similar patients who present with encephalopathy at 10 days after symptom onset.[7,8]

The survey was performed in hospital with active members of the Japan Society of Hepatology and the Japanese Society of Gastroenterology. Patients who meet the diagnostic criteria for FH and LOHF were entered into the survey (Table 1). Besides the diagnostic criteria, patients under 1 year of age and those with alcoholic hepatitis were excluded from the analysis.

The etiology of acute liver failure is classified into five categories: viral infection, autoimmune hepatitis, drug-allergy-induced liver injury, unknown, and indeterminate (Table 2). Patients with viral infection consist of those with hepatitis A virus (HAV),

Table 1 Diagnostic criteria for fulminant hepatitis in Japan according to the Intractable Liver Diseases Study Group of Japan, the Ministry of Health, Welfare and Labour (2003)

Fulminant hepatitis (FH) is defined as hepatitis in which hepatic encephalopathy of coma grade greater than II develops in the patients within 8 weeks after the onset of disease symptoms with highly deranged liver functions showing prothrombin time less than 40% of the standardized values.

FH is classified into two subtypes: the acute typeand subacute type in which the encephalopothy occurs within 10 days and later than 11 days, respecitvely.

Note 1: Patients with chronic liver diseases are excluded from FH, but asymptomatic HBV carriers who develop acute exacerbation are diagnosed with FH.

Note 2: Acute liver failure accompanying no liver inflammation, such as drug or chemical intoxication, microcirculatory disturbance, acute fatty liver of pregnancy, and Reye's syndrome are excluded from FH.

Note 3: The grading of hepatic encephalopathy is based on the criteria from the Inuyama Symposium in 1972.

Note 4: The etiology of FH is based on the criteria from the Intractable Liver Diseases Study Group of Japan in 2002 (Table 2).

Note 5: Patients with no hepatic encephalopathy or encephalopathy of coma grade I, even showing prothrombin time <40% of the standardized values, are diagnosed with severe acute hepatitis. Patients in whom encephalopathy develops between 8 and 24 weeks after disease onset, with prothrombin time <40% of the standardized values, are diagnosed with late onset hepatic failure (LOHF). Both are related to FH, but are regarded differently from FH.

hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis E virus (HEV) and other viruses. Patients with HBV infection are further classified into transient infection and acute exacerbation of HBV carrier status. In 2002, the criteria were modified to define FH due to autoimmune hepatitis and HEV, and the etiology of patients between 1998 and 2001 was re-assessed according to these new criteria.

Demographic features

From 1998 to 2006, 934 patients were enrolled in the surveillance.[9] Among these patients, 856 (432, acute type and 424, subacute type) were classified as having FH and 78 as having LOHF (Table 3). Based on the nationwide epidemiology surveillance, the annual incidence of FH was estimated at 3700 cases in 1972, 1050 cases in 1995, and 429 cases in 2004.[10] About 30% of patients with severe acute hepatitis were presumed to develop hepatic encephalopathy of coma grade II or more.[11]

The male : female ratio was higher for the acute type than subacute type and LOHF. The age of the patients was significantly higher for the subacute type and LOHF than for the acute type. The frequency of HBV carriers was highest for the subacute type and lowest for LOHF. There were many patients with complications, such as metabolic syndrome, malignancy and psychiatric disorders, which preceded the onset of acute liver failure, and most of these patients had received daily medication. This tendency was more obvious in patients with the subacute type and LOHF.

The survival rates of non-liver-transplanted patients were 54% for acute and 24% for subacute type FH, and 15% for LOHF. The prognosis of patients with subacute type FH and LOHF was evidently poor. These annual rates have not improved between 1998 and 2006. When compared to a previous survey,[12] prognosis of FH in acute type patients improved until 1998, although the prognosis remained poor in the subacute type with no liver transplantation during that period (Fig. 1). This improvement was probably achieved by progress in artificial liver support.

Table 2 Criteria for etiology of fulminant hepatitis and late onset hepatic failure

I. Viral infection
  1. HAV: positive for serum IgM anti-HAV
  2. HBV: positive for either serum HBsAg, IgM anti-HBc or HBV DNA
    A. Transient infection: fulfilling either (a) or (b):
      (a) Negative for serum HBsAg before onset of acute liver injury.
      (b) Positive for serum IgM anti-HBc and negative for anti-HBc in serum diluted to 1:200.
    B. Acute exacerbation of carrier status: fulfilling either (a) or (b):
      (a) Positive for serum HBsAg before onset of acute liver injury
      (b) Negative for serum IgM anti-HBc and positive for anti-HBc in the serum diluted to 1:200.
    C. Undetermined: neither (a) nor (b)
  3. HCV: fulfilling either (a) or (b):
    (a) Negative for serum anti-HCV or HCV RNA before onset of acute liver injury.
    (b) Positive for serum HCV RNA and low titer positive for serum anti-HCV core protein.
  4. HEV: positive for serum HEV-RNA
  5. Other virus: e.g. EBV.

II. Autoimmune hepatitis: fulfilling either (a) (b) or (c):
  (a) Diagnosed as definite or probable according to the International Scoring System for autoimmune hepatitis.
  (b) Attenuation of liver injury after glucocorticosteroid administration and/or aggravation of liver injury following withdrawal of glucocorticoid.
  (c) Positive for serum antinuclear antigen and/or serum IgG levels >2 g/dL.

III. Drug-allergy-induced: drugs responsible for liver injury are determined by clinical course of liver injury and/or d-LST.

IV. Unknown: etiology is unknown despite sufficient examinations available.

V. Undetermined: etiology is undetermined because of insufficient examinations.

HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; EBV, Epstein-Barr virus; d-LST, drug-induced lymphocyte stimulation test.

Causes of FH

Viral hepatitis

In Japan, the cause of FH has been identified as HAV, HBV or other viruses in about 50% of patients (Table 4). The causes of acute liver failure differed depending on the disease type. The frequencies of viral infection were 69% and 31% for patients with the acute and subacute types of FH, respectively, and 17% for LOHF patients.

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Table 3 Demographic features of patients with fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006)

		FH		LOHF
	Total ($n = 856$)	Acute type ($n = 432$)	Subacute type ($n = 424$)	($n = 78$)
Men/women	431/423	228/203	197/226	33/45
Age (years; mean ± SD)	48 ± 17	46 ± 16	49 ± 17**	53 ± 15**
HBV carrier rate (%)	14	12	16*	7***
Complications (%)	39	35	44*	49*
History of medication (%)	46	41	51**	54*
Survival rate (no LT) (%)	40	54	24**	15**
Survival rate (LT) (%)	77	73	79	81

*$P < 0.05$; **$P < 0.01$ versus acute type; ***$P < 0.05$ versus subacute type.
HBV, hepatitis B virus; LT, liver transplantation.

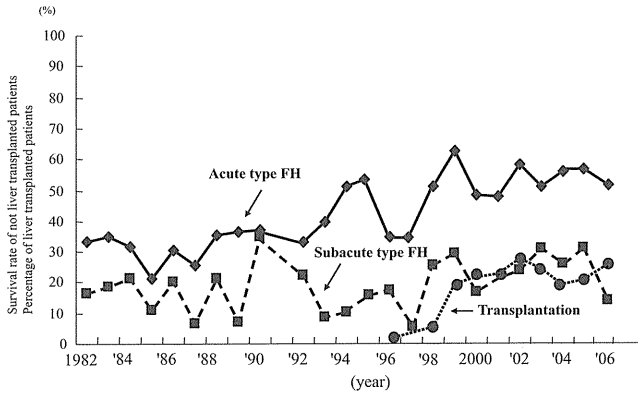

Figure 1 Survival rate of not liver transplanted patients with fulminant hepatitis(FH) and percentage of liver transplanted patients.

Table 4 Percentage etiology of fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006)

		FH		LOHF
	Total ($n = 856$)	Acute type ($n = 432$)	Subacute type ($n = 424$)	($n = 78$)
Viral infection	51	69	31	17
HAV	6	11	1	1
HBV	42	56	27	13
(Transient infection)	(25)	(40)	(9)	(5)
(Carrier)	(13)	(11)	(16)	(4)
(Undetermined)	(4)	(6)	(2)	(4)
HCV	1	1	1	1
HEV	1	1	1	0
Other virus	1	1	1	1
Autoimmune hepatitis	7	2	12	18
Drug-allergy-induced	10	8	13	15
Unknown	30	18	42	47
Indeterminate	3	3	3	3

HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus.

Infection with HAV was found in 6% of patients with FH and frequently observed in the acute type. As annual incidence of acute hepatitis A has declined over the past decade,[13] so too has the incidence of FH. However, as the overall immunity of the Japanese population to hepatitis A is only 12%[14] and is decreasing gradually as in other non-endemic areas, the increasing risk of future outbreaks of acute hepatitis A is probable. With regard to the severity of hepatitis A, age, sex, and drug toxicity have been identified as potential contributing factors.[15] HAV susceptibility and the risk of severity have likely increased recently.

In most of the patients, viral infections were due to HBV. HBV infection was found in 42% of patients with FH and 13% of those with LOHF. Among these, transient HBV infection was more frequent than acute exacerbation of HBV carrier status. Transient HBV infection was more frequent in the acute type (40%) than subacute type (9%) of FH, whereas the frequency of HBV carrier status was greater in the subacute type (16%) than in the acute type (11%). Annual incidence of FH due to HBV infection, both in transient HBV infection and acute exacerbation of HBV carrier status, has declined over the past decade. The routes of transmission of HBV indicate that, at present, sexual transmission from HBV carriers is a major route for FH. The preventive administration of HBV hyperimmune globulin and vaccination against HBV of neonates born to HBV-carrier mothers has been practiced nationwide since 1985 in Japan.[16] Therefore, the HBV carrier rate in the population has significantly decreased, and as a result, a marked decrease in the incidence of FH caused by HBV is expected.

Reactivation of HBV is a well-recognized complication in patients with chronic HBV infection who are undergoing cytotoxic chemotherapy or immunosuppressive therapy. HBV reactivation can be clinically severe and result in death from acute liver failure. Among acute exacerbation of HBV carrier status in the survey, HBV reactivation has been increasingly observed in patients with hematological malignancies. Furthermore, among the 12 patients with HBV reactivation, six with serological evidence of resolved hepatitis B [without hepatitis B surface antigen (HBsAg), but with antibody to hepatitis B core antigen (anti-HBc) and/or antibody to HBsAg (anti-HBs) in serum] developed reactivation with reappearance of HBsAg in serum. Most of these patients had received rituximab and coriticosteroid. Recently, combination therapy with rituximab and corticosteroid has been identified as a risk factor for HBV reactivation in HBsAg-negative patients with malignant lymphoma.[17,18] A study in Japan has revealed that 22% of *de novo* hepatitis B and that caused by HBV reactivation from resolved

hepatitis developed into fulminant hepatic failure, and mortality was 100%.[19] This problem deserves careful attention, because HBsAg-negative, anti-HBc-and/or anti-HBs-positive patients, which account for 20–25% of hospitalized patients in Japan, represent a high-risk group.[20]

HCV infection is rare in the etiology of patients with FH and LOHF. HCV infection was found in 1% of patients with FH, independent of the disease type. Reactivation of HCV as a cause of acute liver failure following chemotherapy has been reported.[21] However, none of these patients were found in the survey.

HEV infection was found in 1% of FH patients. HEV is a common cause of acute hepatitis in endemic areas, such as South Asia, Africa and South America.[22] The virus is now also known to exist indigenously in Japan, and can contribute to acute liver disease.[23,24] In Japan, the zoonotic transmission from pigs, wild boar and deer, either food-borne or otherwise, is the cause of HEV infection in non-endemic areas.[24,25] As for the geographical distribution of clinical HEV infection in Japan, it has been reported that there was wide variation with a higher prevalence in the northern part of Japan (Hokkaido Island and the northern part of mainland Honshu).[26] In the survey, two-thirds of the patients were from this area. Moreover, most of the patients were elderly men and there were no pregnant women, who have the highest attack rate of the virus in endemic areas.

In the survey, Epstein–Barr virus, cytomegalovirus, herpes simplex virus, human herpesvirus type-6 and parvovirus were infrequent causes of other forms of viral hepatitis.

Autoimmune hepatitis

Although autoimmune hepatitis is a chronic disease, an acute presentation occurs in approximately 22% of patients, and an even smaller number present with acute liver failure.[27] In the survey, autoimmune hepatitis was found in 7% of patients with FH and 18% of those with LOHF, respectively. In 2001, FH due to autoimmune hepatitis was recognized in Japan, because there were patients with non-HAV/HBV FH in which IgG levels were >2 g/dL, with positive antinuclear antigen in the serum. Although the diagnosis generally relies on the presence of serum autoantibodies, higher IgG levels (>2 g/dL), liver histology (if available), and response to corticosteroid therapy, the diagnosis of acute-onset autoimmune hepatitis is often difficult. The serum gammaglobulin or IgG concentrations are often lower than those in patients with chronic hepatitis.[28]

Drug-allergy-induced liver injury

Formation of toxic reactive metabolites has been suggested as a potential mechanism for causing idiosyncratic drug-induced liver injury.[29] Drug-allergy-induced liver injury was seen in 13% of patients with subacute type FH and in 15% of those with LOHF. The diagnosis relied mostly on the clinical course or drug-induced lymphocyte stimulation test (D-LST). Numerous types and classes of drugs have been implicated. Anti-tuberculosis agents (isoniazid, rifampicin, ethambutol and pyrazinamide), nonsteroidal anti-inflammatory drugs (loxoprofen, lornoxicam and acetaminophen), anti-cancer agents (tegafur, UFT and flutamide), drugs for metabolic syndrome (allopurinol and acarbose), and various herbal and natural remedies were the probable causative agents in the survey.

Table 5 Survival rates and etiology of patients with fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006)

		FH		LOHF
	Total ($n = 678$)	Acute type ($n = 369$)	Subacute type ($n = 309$)	($n = 62$)
Viral infection	45	55	23*	36*
HAV	74	77	40	100
HBV	39	50	18*	38
(Transient infection)	(51)	(56)	(32*)	(33)
(Carrier)	(22)	(35)	(13*)	(67)
(Undetermined)	(23)	(33)	(0)	(0)
HCV	67	75	60	0
HEV	60	100	33	—
Other virus	60	50	67	0
Autoimmune hepatitis	21	25	21	18
Drug allergy-induced	42	58	29*	0*
Unknown	36	54	26*	10*
Indeterminate	28	36	14	0

*$P < 0.05$ versus acute type.
HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus.

Unknown etiology

The etiology was unknown in 42% and 47% of patients with subacute type FH and LOHF, respectively. Although the roles of GB virus C (GBV-C)/hepatitis G virus (HGV) and transfusion transmitted virus (TTV) have been discussed, in this survey, neither GBV-C/HGV or TTV appeared to be a major cause of FH. It is possible that the patients with drug-allergy-induced liver injury were contaminated with those of unknown etiology, because the ratio of medication history was high in these patients. The relationship between daily dose of oral medication or medication with significant hepatic metabolism and idiosyncratic drug-induced liver injury has been reported.[30,31] The higher numbers of patients with complications and daily medication in the survey support this evidence. Furthermore, HEV infection needs further investigation, because serum HEV RNA and IgM antibody to HEV were measured less in the survey.

Prognosis

The prognosis of patients with FH and LOHF differed depending on the etiology (Table 5). It was excellent in patients with HAV infection: the survival rate was 77% and 40% in patients with acute and subacute types of FH, respectively, and 100% in those with LOHF. In contrast, the prognosis was especially poor in HBV carriers who showed acute exacerbation. The survival rates of acute and subacute types of FH were 35% and 13%, respectively. It is noteworthy that none of the patients with HBV reactivation from resolved hepatitis B after rituximab and corticosteroid combination therapy survived. In contrast, the survival rate was 56% in acute type FH and 32% in subacute type in patients with transient HBV infection. The prognosis was poor in autoimmune hepatitis independent of disease type. Prognosis was also poor in patients

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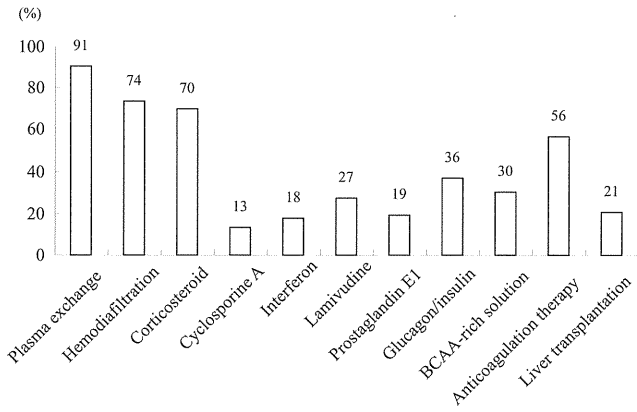

Figure 2 Percentage incidence of therapies performed for fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006). BCAA, branched-chain amino acid.

with subacute type FH and LOHF caused by drug-allergy-induced liver injury, and in those of the unknown etiology.

Complications

Complications that occurred during the course of acute liver failure also seemed to affect patient prognosis. Disseminated intravascular coagulation, renal failure and bacterial infection were found as complications in >30% of patients. Brain edema, gastrointestinal bleeding and congestive heart failure were seen in about 30%, 20% and 10%, respectively. Any of these complications significantly decreased survival rate. Furthermore, the number of these complications influenced prognosis.

Management

Specific therapies

The frequency of antiviral therapy with lamivudine has increased since 1998. As antiviral agents, lamivudine and interferon have been used in 27% and 18% of patients with FH and LOHF, respectively, between 1998 and 2006 (Fig. 2). Lamivudine has been used in 67% of patients with HBV-related FH or LOHF. Lamivudine has been reported to be efficacious for acute liver failure.[31,32] Recently, another guanosine nucleoside analog, entecavir, has been administered more frequently.[33] A preliminary study of entecavir for acute liver failure has revealed that the agent beneficially affects disease course. Lamivudine therapy is more efficacious when started early in acute liver failure. However, in the case of HBV reactivation from HBsAg-negative patients, it is difficult to prevent development of liver failure, even when lamivudine is administered after the onset of hepatitis. Two study groups in Japan have proposed guidelines for prevention of immunosuppressive-therapy- or chemotherapy-induced HBV reactivation. These guidelines recommend that patients with resolved infection should be routinely monitored for liver function and HBV DNA levels during and after chemotherapy, and antiviral therapy should be administered immediately when HBV DNA increases above the detection levels.

Corticosteroids were administered in 70% of patients with FH and LOHF. Steroid pulse therapy, methylprednisolone at a daily dose of 1 g injected intravenously, was administered to attenuate liver necrosis by suppressing excessive immune response. The efficacy of corticosteroids for improving the prognosis of acute liver failure is still obscure. Some randomized controlled trials have shown that corticosteroids provide no benefit overall in acute liver failure.[34] However, FH due to autoimmune hepatitis might be a candidate for therapy.[35] Anticoagulant therapy was performed in 56% of patients with FH and LOHF. Antithrombin III concentrate and protease inhibitor compounds such as gabexate mesylate and nafamostat mesylate were used as anticoagulants. They were effective for inhibition of disseminated intravascular coagulation and microcirculatory disturbance due to sinusoidal fibrin deposition. Glucagon/insulin, branched-chain amino acid-rich solution, cyclosporine A and prostaglandin E1 therapy was administered less frequently, and the frequency decreased compared to that in patients in the previous survey between 1995 to 1997.

Methods of liver support

In Japan, powerful artificial liver support with plasmapheresis and hemodiafiltration plays a central role in the treatment of acute liver failure. Plasmapheresis and hemodiafiltration were performed in 91% and 74% of patients with FH and LOHF, respectively (Fig. 2). In the late 1990s, hemodiafiltration therapy was developed and plasma exchange combined with hemodiafiltration therapy became popular. The increased frequency of this combination therapy in the 1990s could be implicated in the tendency for the survival rate to increase for acute type FH (Fig. 1). The effect of plasmapheresis on survival from acute liver failure has been difficult to determine. However, these support systems are efficacious for helping patients to remain in good condition until sufficient regeneration of the liver can be obtained, or liver transplantation can be performed. Recently, more powerful hemodiafiltration using large buffer volumes[36] or on-line hemodiafiltration[37] has been developed and has shown greater efficacy for improving hepatic coma.

Liver transplantation

Despite significant advances in critical care and an improved understanding of the pathophysiology of acute liver failure, the mortality rate remains high. Liver transplantation is the only life-saving treatment available beyond the supportive care of a critical unit. In Japan, living donors have been used because of the insufficiency of organ donation since 1988. Living donor liver transplantation was performed in 17% of patients with FH and LOHF between 1998 and 2006, and the frequency in those patients was significantly greater in the subacute type (21%) than in the acute type (13%). Recently, these frequency ratios have been almost steady (Fig. 1). The survival rates were 77% and 81% in patients with FH and LOHF, respectively, and there was no difference in the rates among the disease types. Patient and graft survival rates were 94% and 87% at 1 year, and 91% and 81% at 5 years, respectively. There was no significant difference in patient and graft survival according to etiology.[38]

Appropriate judgment to move forward to liver transplantation is the most important step. The indications for liver transplantation

in cases of FH are determined according to the 1996 Guidelines of the Acute Liver Failure Study Group of Japan. Re-evaluation of the guidelines has revealed that the accuracy in patients not receiving liver transplantation was 68% and 78% in acute and subacute types of FH, respectively, and 84% among those with LOHF.[39] The sensitivity and specificity of the assessment in patients with acute and subacute types were very low. To improve this situation, new guidelines for using a scoring system have been proposed by the Intractable Hepato-biliary Disease Study Group of Japan.[40] By using these guidelines, the accuracy in patients not receiving liver transplantation was increased to 75% and 87% in acute and subacute types of FH, respectively.

Experimental methods of liver support

To improve the prognosis of acute liver failure, advances in the treatment for liver regeneration are urgently needed. Hepatocyte growth factor (HGF) acts as a stimulator of liver regeneration, as well as an anti-apoptotic factor. We have started a clinical trial to examine the effects of recombinant human HGF (rhHGF) in patients with FHor LOHF, and in the four patients with FH or LOHF enrolled in this study; repeated doses of rh-HGF did not produce any severe side effects. Although two patients were rescued in this study, evaluation of this therapeutic agent is still under investigation.[41]

Several clinical trials of bone marrow cell infusion in patients with liver cirrhosis have shown clinical improvement. A clinical trial of autologous bone marrow infusion for patients with advanced liver cirrhosis due to chronic HBV infection has shown clinical improvement with no serious adverse events.[42] The recent discovery of pluripotent stem cells has yielded a new cell type for potential application in regenerative medicine. Strategies to achieve high levels of hepatocyte survival and the development of methods to engineer a functional liver system *in vivo* are expected in the future.

Conclusion

In Japan, the incidence of FH has decreased gradually and the clinical characteristics of patients and the therapeutic approach have changed in the past decade. The prognosis differs in patients with FH and LOHF depending on the disease type and etiology. HBV is the major cause of FH in Japan. Recently, careful attention has been necessary because of an increase in HBV reactivation from resolved hepatitis B. Despite careful investigation, a significant group with FH of unknown origin remains and needs further investigation. Living donor liver transplantation is the only life-saving treatment available beyond the supportive care of a critical unit. Artificial liver support systems are efficacious while waiting until the native liver regenerates or a donor is found. New therapeutic modalities are required to regenerate the liver, in particular, for the subacute type of FH.

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Original article

De novo activation of HBV with escape mutations from hepatitis B surface antibody after living donor liver transplantation

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Background: *De novo* activation of HBV occurs after liver transplantation from hepatitis B surface antigen (HBsAg)-negative and hepatitis B core antibody (anti-HBc)-positive donors, even under hepatitis B immunoglobulin (HBIG) prophylaxis. One reason for the activation of HBV is the emergence of HBV with escape mutations from hepatitis B surface antibody (anti-HBs). The aim of this study is to clarify the clinical features for *de novo* activation of HBV with anti-HBs escape mutations after liver transplantation.
Methods: Clinical features of 75 patients who received HBIG prophylaxis >6 months after liver transplantation with liver grafts from anti-HBc-positive donors were retrospectively analysed.
Results: Among the 75 recipients, 19 (25%) developed *de novo* activation of HBV. Of the 19 recipients, the emergence of HBV with anti-HBs escape mutations was confirmed in 7 patients. The rate of *de novo* activation of HBV with anti-HBs escape mutations was 12% at 5 years. Sequence analysis revealed mutations in the common 'a' determinant region of the surface gene, including G145R, G145A and Q129P, in HBsAg. Administration of entecavir immediately after the occurrence of *de novo* HBV activation resolved hepatitis and induced clearance of serum HBsAg and HBV DNA in all four patients receiving entecavir.
Conclusions: Escape mutations from anti-HBs caused *de novo* activation of HBV under HBIG prophylaxis after liver transplantation. Early administration of entecavir was effective on *de novo* activation of HBV with anti-HBs escape mutations.

Introduction

Most individuals who are negative for hepatitis B surface antigen (HBsAg) but positive for hepatitis B core antibody (anti-HBc) – which is indicative of resolved hepatitis B – have persistent viral infection in the liver [1,2]. We previously demonstrated that latent HBV infection is accompanied by ongoing viral replication in the liver but not in the serum or lymphatic cells of healthy anti-HBc-positive liver transplant donors [3,4]. It is possible for latently infected HBV to be transmitted from anti-HBc-positive donors to recipients via liver grafts and reactivated under the immunosuppressive conditions imposed after liver transplantation. This reactivation is called *de novo* activation of HBV [5–7].

To prevent *de novo* activation of HBV after liver transplantation, hepatitis B immunoglobulin (HBIG) had been widely used as a prophylaxis post-surgery [7–9], although lamivudine with or without HBIG has recently become the standard prophylaxis [10,11]. Even under HBIG prophylaxis, occurrence of *de novo* activation of HBV has been reported [8,12,13]. Recently, we reported that *de novo* hepatitis B occurred in 24% of HBV-naive recipients who received liver grafts from anti-HBc-positive donors [13]. Among these cases, one of the most important factors associated with HBV activation was found to be the emergence of HBV with escape mutations from hepatitis B surface antibody (anti-HBs). Escape mutations from anti-HBs occur in the common 'a' determinant region of the surface gene, which is a highly conformational region of the HBsAg protein. Mutations in and around the 'a' determinant region have been shown to alter the antigenicity of the HBsAg protein; consequently, anti-HBs fails to neutralize HBV

[14–16]. Anti-HBs escape mutations have been found in patients vaccinated for HBV [17,18], in patients with chronic hepatitis B [19,20] and in liver transplant recipients after HBIG administration [21,22]. The clinical significance of the anti-HBs escape mutant HBV has been well-analysed in patients after HBV vaccination. The prevalence of anti-HBs escape mutants after HBV vaccination was reported to have increased from 7.8% in 1984 to 19.6% in 1989; after a 1994 survey, prevalence was reported to be 28.1% [18]. Commonly reported mutations in HBsAg with the potential to escape neutralization by vaccine-induced antibody in patients after HBV vaccination include G145R, D144A, P142S, K141E, Q129H, I/T126N/A and M133L [18,23]. By contrast, the clinical features of anti-HBs escape mutants after liver transplantation under HBIG prophylaxis have not been well-analysed.

Treatment strategies for HBV with anti-HBs escape mutations have not been clarified. At present, several nucleoside analogues such as lamivudine, adefovir and entecavir are available for the treatment of chronic hepatitis B [24]. Among them, entecavir, a carbocyclic analogue of 2′-deoxyguanosine, has been shown to have higher efficacy and lower rates of resistance than lamivudine for patients with chronic hepatitis B [24]; therefore, entecavir is now used as a first-line therapy in the treatment of chronic hepatitis B worldwide. However, the efficacy of nucleoside analogues for HBV with escape mutations from anti-HBs is unknown.

The aim of this study was to clarify the clinical features of *de novo* activation of HBV with escape mutations from anti-HBs under HBIG prophylaxis after liver transplantation.

Methods

Patients

We retrospectively analysed the medical records of 157 patients who underwent living donor liver transplantation (LDLT) using liver grafts from HBsAg-negative but anti-HBc-positive donors from July 1995 to August 2008 (Figure 1A). Of these, 57 recipients were excluded from our study because their sera were pre-operatively positive for HBsAg and/or HBV DNA. An additional 25 patients were also excluded from the study because of the short duration (<6 months) of their follow-up in our hospital. Accordingly, 75 patients with a follow-up period of >6 months were enrolled in this study. The study protocol was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine (Kyoto, Japan), and all patients provided informed consent.

Prophylaxis with HBIG and immunosuppressive protocol

HBIG monotherapy was given to all recipients with grafts from anti-HBc-positive donors, as reported previously [7]. The first application of HBIG at a dose of 200 IU/kg body mass was administered during the anhepatic phase of LDLT, and 100 IU/kg of HBIG was administered, if required, to maintain serum anti-HBs titres at >500 IU/l during the first post-operative month. Subsequently, HBV serological markers were examined at monthly intervals after the transplant operation, and 1,000 IU of HBIG was periodically administered to maintain serum anti-HBs titres at >200 IU/l throughout the follow-up period.

The standard immunosuppression protocol comprised tacrolimus and low-dose steroid therapy. The target whole blood lower level for tacrolimus was 10–15 ng/ml during the first 2 weeks, 10 ng/ml thereafter and 5–8 ng/ml starting from the second month. Steroid therapy was initiated at a dose of 10 mg/kg of prednisolone before graft reperfusion, then tapered down from 1 mg/kg per day on the first day to 0.3 mg/kg per day until the end of the first month, followed by 0.1 mg/kg per day until the end of the third month. After that, steroid administration was terminated.

Diagnosis of *de novo* activation of HBV

De novo activation of HBV was diagnosed when HBsAg and HBV DNA became positive in the serum of the liver transplant recipient. Serological HBV markers, including HBsAg, anti-HBs, anti-HBc, hepatitis B e antigen (HBeAg) and antibodies to HBeAg (anti-HBe), were measured by chemiluminescent enzyme immunoassay (CLEIA; Fuji Rebio, Tokyo, Japan). Serum HBV DNA titre was analysed using a commercial PCR assay (Amplicor HBV Monitor; Roche, Branchburg, NJ, USA).

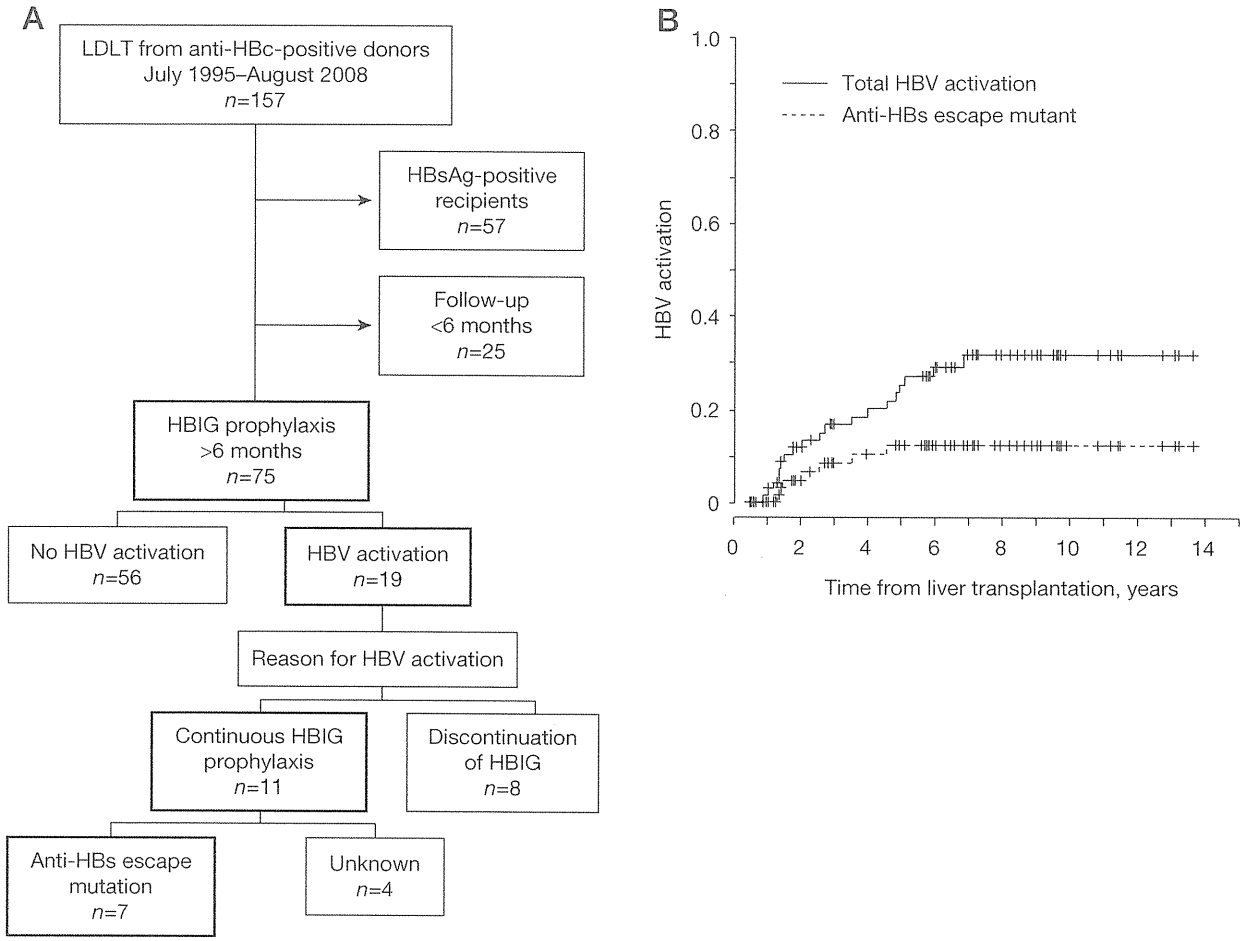
PCR amplification of HBV DNA and sequencing of the surface gene

Serum samples were obtained at the diagnosis of *de novo* activation of HBV for the analysis of HBV DNA sequencing. Preparation of DNA samples and detection of HBV genomes by PCR have been described previously [3,13]. The nucleotide sequence spanning the S region was amplified by PCR using specific primers, 5′-TGCCCTTGGATAAAGGCATT-3′ and 5′-AAGTTAAGGGAGTAGCCCCA-3′, followed by direct sequencing analyses using primers 5′-CCTGCTGGTGGCTCCAGTTC-3′ and 5′-AAGTTAAGGGAGTAGCCCCA-3′.

Statistical analysis

Baseline characteristics were tabulated and compared between patients with activation of HBV with anti-HBs escape mutations and patients without HBV activation (Table 1). For continuous variables, medians and ranges are given, and the data were analysed by the Wilcoxon rank-sum test. For categorical variables, counts

Figure 1. Flow diagram and Kaplan–Meier estimates of *de novo* activation of HBV after LDLT



(A) Flow diagram showing *de novo* activation of HBV after living donor liver transplantation (LDLT) from hepatits B core antibody (anti-HBc)-positive donors. (B) Kaplan–Meier estimates of the rate of patients who showed *de novo* activation of HBV after LDLT from anti-HBc-positive donors. The total rate and rate of activation of HBV with hepatitis B surface antibody (anti-HBs) escape mutations are shown. HBIG, hepatitis B immunoglobulin; HBsAg, hepatitis B surface antigen.

are given, and the data were analysed by the χ^2 test. The rates of patients who showed HBV activation after LDLT were estimated using the Kaplan–Meier method. $P<0.05$ was considered significant.

Results

De novo activation of HBV in recipients from anti-HBc-positive donors

Among the 75 recipients who received HBIG prophylaxis >6 months after LDLT with liver grafts from anti-HBc-positive donors, 19 (25%) patients developed *de novo* activation of HBV (Figure 1A). The rate of HBV activation estimated by the Kaplan–Meier method was 3% at 1 year, 17% at 3 years, 25% at 5 years, and 29% at 10 years (Figure 1B). Of the 19 recipients with HBV activation, 8 had HBV activation due to transient discontinuation of HBIG (Figure 1A). In the remaining 11 patients with HBV activation, despite continuous HBIG prophylaxis, the emergence of HBV with anti-HBs escape mutations was confirmed in 7 patients, including 2 patients who were described in our previous report [13]. The rate of *de novo* activation of HBV with anti-HBs escape mutations estimated by the Kaplan–Meier method was 8% at 3 years and 12% at 5 years (Figure 1B). The other four recipients, in whom the reason for HBV activation was unknown, were previously reported by us [13].

Clinical features of patients with *de novo* activation of HBV with anti-HBs escape mutations

To clarify the characteristics of patients with *de novo* activation of HBV with anti-HBs-escape mutations, the clinical features of recipients with HBV with anti-HBs escape mutations ($n=7$) were listed and compared

Table 1. Clinical features of patients with *de novo* activation of HBV with anti-HBs escape mutation, of those without HBV activation after liver transplantation and also of donors

Characteristic	Activation of HBV with anti-HBs escape mutations (n=7)	No HBV activation (n=56)	P-value
Recipient			
Age, years	17 (0–67)	12 (0–62)	0.433[a]
Male/female	3/4	21/35	1.000[b]
Primary disease			
Cholestatic diseases	4 (57)	31 (55)	-
Hepatocellular diseases	0	8 (14)	-
Neoplastic diseases	1 (14)	6 (11)	-
Acute liver failure	0	1 (2)	-
Metabolic diseases	0	3 (5)	-
Retransplantation	1 (14)	6 (11)	-
Other	1 (14)	1 (2)	-
HBV markers before LDLT			
Anti-HBs-positive	3 (43)	18 (32)	0.677[b]
Anti-HBe-positive	0	7 (13)	0.581[b]
Anti-HBc-positive	0	17 (30)	0.175[b]
Donor			
Age, years	42 (34–55)	45 (24–65)	0.200[a]
Male/female	3/4	31/25	0.694[b]
Anti-HBs-positive	7 (100)	44 (79)	0.329[b]
Anti-HBe-positive	4 (57)	38 (68)	0.419[b]
Follow-up period, months	86 (48–151)	94 (12–180)	0.646[a]

Qualitative variables are displayed as n (%) and quantitative variables expressed as median (range) for non-normally distributed variables. [a]Wilcoxon rank-sum test. [b]χ^2 test. Anti-HBc, heptatitis B core antibody; anti-HBe, hepatitis B e antibody; anti-HBs, hepatitis B surface antibody; LDLT, living donor liver transplantation.

Table 2. Clinical features of seven patients with *de novo* activation of HBV with anti-HBs escape mutation after liver transplantation

Patient number	Age, years[a]	Sex	Months from LDLT to HBV activation	Anti-HBs titre before HBV activation, IU/l	At the time of HBV activation			
					Anti-HBs titre, IU/l	HBsAg, COI	Serum HBV DNA, copies/ml	Peak ALT, IU/l
1	12	F	16.8	418.4	275.2	>2,000	$>10^{7.6}$	410
2	22	M	54.9	27.8	16.6	60.4	$10^{6.6}$	1,300
3	26	F	17.9	284.5	82.6	10.2	$>10^{7.6}$	81
4	70	F	42.2	44.0	17.9	668.8	$10^{7.1}$	204
5	59	M	24.1	188.8	105.4	21.3	$10^{3.5}$	131
6	18	F	30.6	96.9	157.4	6.9	$10^{4.5}$	153
7	2	M	15.7	218.8	74.3	187.3	NE	111

[a]Age at HBV activation. ALT, alanine aminotransferase; anti-HBs, hepatitis B surface antibody; COI, cutoff index; F, female; HBsAg, hepatitis B surface antigen; LDLT, living donor liver transplantation; M, male; NE, not examined.

with those of recipients without HBV activation (n=56; Table 1). The two groups of patients did not differ significantly by age, sex or serological markers for HBV before LDLT with regard to either recipients or donors. Of note, all seven patients with *de novo* activation of HBV with anti-HBs escape mutations were negative for anti-HBc pre-operatively, and no anti-HBc-positive recipients (n=17) developed *de novo* activation of HBV.

The details of the clinical features of the seven patients who developed *de novo* activation of HBV with anti-HBs-escape mutations are summarized in Table 2. In the seven patients, serum HBsAg and HBV DNA became positive 15.7–54.9 months (median 24.1 months) after LDLT. Serum anti-HBs titres were maintained at 27.8–418.4 IU/l before HBV activation by HBIG administration. At the time of HBV activation, all patients were positive for anti-HBs, despite being positive for serum HBV DNA and HBsAg, when *de novo* hepatitis B was diagnosed. The genotype of HBV in all seven patients was C, which is the major genotype in Japan [25]. All patients showed high serum ALT levels. Five of the seven patients received tacrolimus only for immunosuppression at the time of HBV activation, patient number 3 had tacrolimus with

prednisolone and mycophenolate mofetil, and patient number 4 received cyclosporine and prednisolone.

Sequence analysis of serum HBV DNA

Results of the sequence analysis of serum HBV DNA in these seven patients are shown in Figure 2. We focused our analysis on the immunodominant loop encompassing amino acid (aa) 101–163 of the S protein, which includes the 'a' determinant region (aa 124–147), the major target of neutralizing anti-HBs antibodies due to its exposure at the surface of viral particles [14,16,26]. Sequencing of the S gene revealed the presence of mutations in the immunodominant loop in all patients. These mutations within the S protein led to G145A substitution in patient number 1, G145R substitution in patients numbered 2, 3, 4, 6 and 7, and Q129P substitution in patient number 5 (Figure 2B). The mutations at aa 145 or aa 129 in HBsAg are known to be escape mutations from anti-HBs [9,16,18,22]. Several other mutations with amino acid substitutions, whose roles have not yet been clarified, were found at aa 101, aa 103, aa 110, aa 113, aa 114, aa 120, aa 126, aa 143, aa 155 and aa 161.

Treatment for *de novo* activation of HBV with anti-HBs escape mutations

Entecavir treatment (0.5 mg) was started for four patients (numbers 1–4) immediately after the diagnosis of *de novo* activation of HBV with anti-HBs-escape mutations. After the administration of entecavir, serum HBsAg and HBV DNA promptly decreased and became undetectable at 2.5, 3.3, 6 and 2.5 months after the start of entecavir treatment, respectively (Figure 3 and Table 3). Serum ALT levels also decreased in association with the decrease in serum HBV DNA. After confirming the stable negativity of HBsAg, entecavir treatment was stopped in three patients (numbers 1–3) at 5.8, 5.9 and 9.9 months after beginning the treatment, respectively. Thereafter, serum HBsAg and HBV DNA remained negative during the follow-up periods of 22.2, 24.7 and 20.6 months after withdrawal of entecavir, respectively (Table 3). Entecavir administration was continued for patient number 4 at the time of the analysis for this study because the patient wanted to continue the treatment. Patients numbered 5 and 6 received early administration of lamivudine after the diagnosis of *de novo* activation of HBV. However, they did not achieve serum HBV clearance by 26.6 and 4.6 months, respectively, at which time adefovir was added. Even after treatment with a combination of lamivudine and adefovir for 33.2 months, patient number 5 remained chronically HBsAg-positive. In patient number 6, serum HBsAg and HBV DNA became negative at 9.5 months after adefovir administration. Patient number 7 who did not receive nucleoside analogue treatment for hepatitis B developed chronic hepatitis B as confirmed by liver histology.

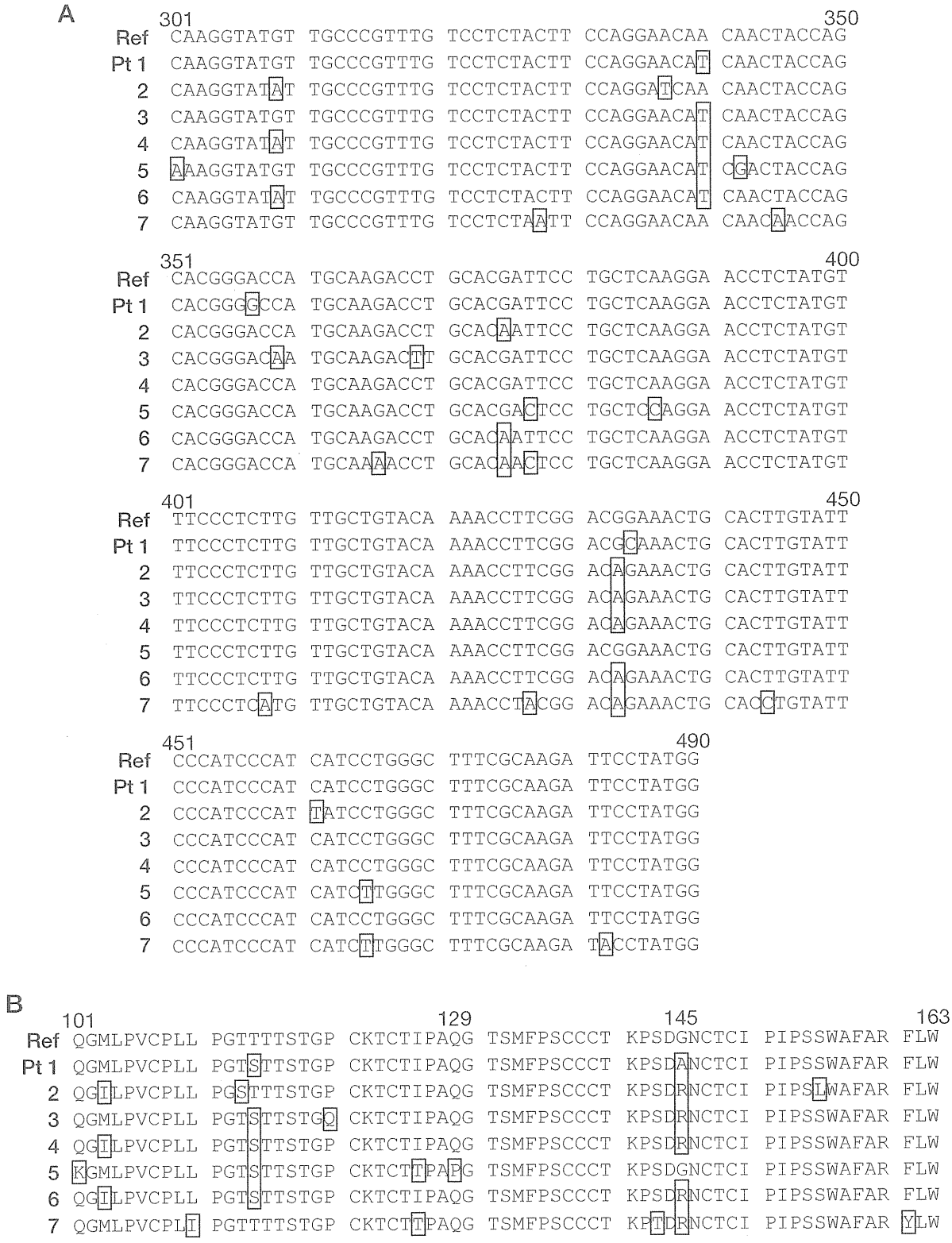
Discussion

In this report, we demonstrated the clinical features of *de novo* activation of HBV with anti-HBs-escape mutations under HBIG prophylaxis after liver transplantation. The rate of *de novo* activation of HBV with anti-HBs escape mutations was 12% at 5 years. No significant difference of baseline characteristics between patients with *de novo* activation of HBV with anti-HBs-escape mutations and patients without HBV activation was identified, but all patients who had activation of anti-HBs escape mutant HBV were pre-operatively anti-HBc-negative. Early entecavir treatment was very effective for patients with *de novo* activation of HBV with anti-HBs escape mutations and the treatment induced complete clearance of serum HBsAg and HBV DNA and resulted in sustained negativity for HBsAg even after the termination of entecavir treatment.

Two reasons for *de novo* activation of HBV after LDLT from anti-HBc-positive donors were revealed in this study: discontinuation of HBIG and emergence of an anti-HBs escape mutant. However, the reason for *de novo* HBV activation in four patients is still unknown. HBV activation by HBIG discontinuation is preventable by careful follow-up to reduce non-compliance of HBIG use. The most important reason for HBV activation is emergence of anti-HBs escape mutations under HBIG prophylaxis, because it is unpredictable and difficult to prevent. In the present study, no anti-HBc-positive patients developed *de novo* activation of HBV with anti-HBs-escape mutations. The reason for this is also unknown, but we expect that individuals with resolved hepatitis B have memory T-cells and various antibodies for HBV, including antibodies against PreS1 and PreS2 as well as anti-HBs, and the T-cells and antibodies could inhibit the proliferation of HBV with anti-HBs-escape mutations.

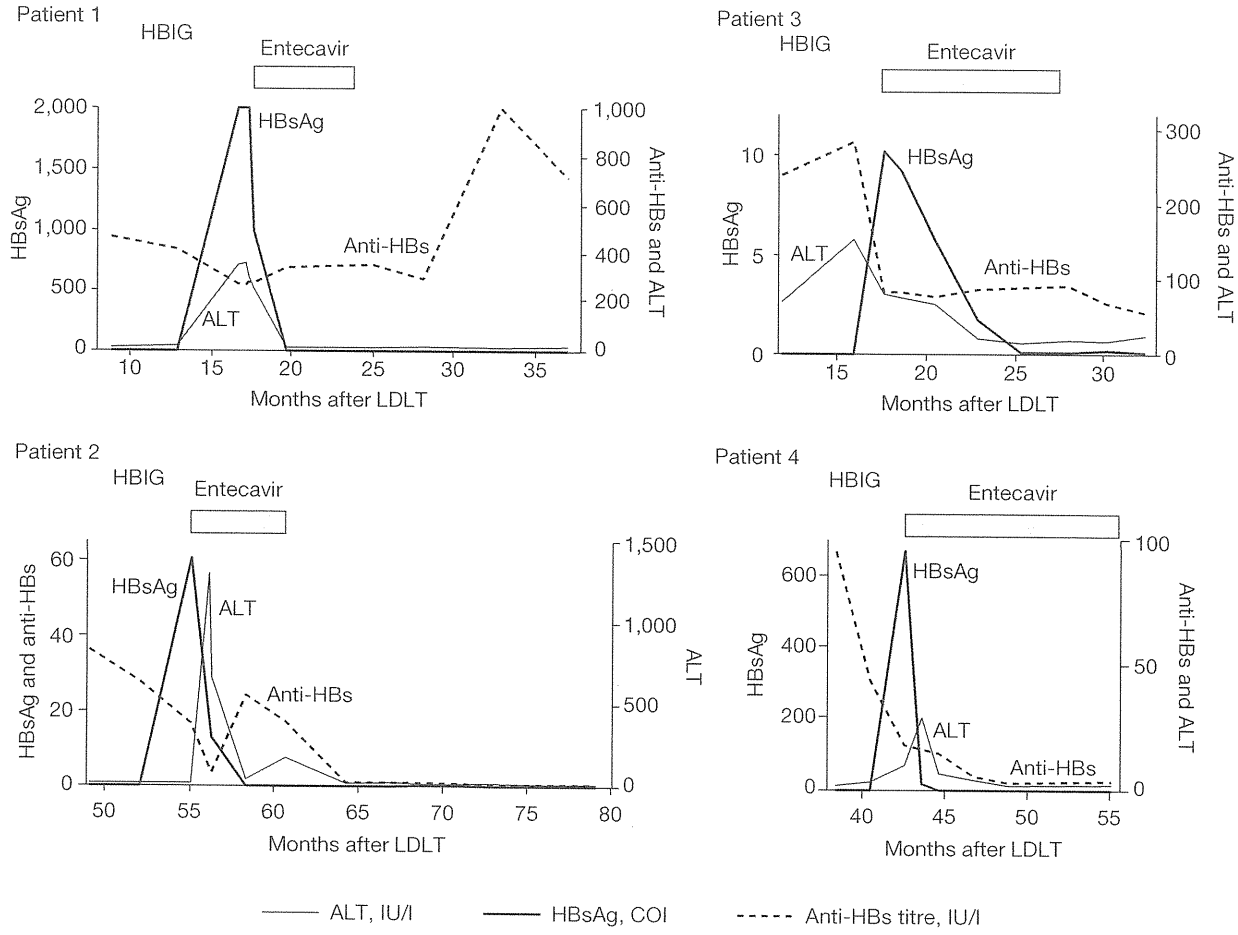
A characteristic serological feature at the onset of *de novo* activation of HBV with anti-HBs-escape mutations was positivity of anti-HBs at the time of HBV DNA appearance in the serum. Because anti-HBs cannot bind HBsAg that has anti-HBs escape mutations [16], HBV will increase even in the presence of anti-HBs. Therefore, we must be cautious to the development of hepatitis B due to HBV with anti-HBs-escape mutations, even when serum anti-HBs titre is maintained at a high level by HBIG administration. A regular evaluation of serum HBsAg and/or HBV DNA is recommended. Duration from liver transplantation to activation of anti-HBs-escape mutants were 15.7–54.9 months in this study. The previous study reported that activation of HBV with

Figure 2. DNA and amino acid sequences from seven patients with anti-HBs escape mutations



(A) DNA sequences between nucleotide 301 and 490 of the *S* gene and (B) amino acid sequences between positions 101 and 163 of the S protein of HBV from seven patients (Pt) with hepatitis B surface antibody (anti-HBs) escape mutations. The sequences are aligned with HBV genotype C (subtype adr) reference sequence (Ref; AB033550 [32]). Boxes indicate the positions showing differences from the Ref.

Figure 3. Clinical course of four patients who received entecavir treatment for *de novo* activation of HBV with anti-HBs escape mutations



The administration of hepatitis B immunoglobulin (HBIG) is shown as arrows, and treatments with entecavir are indicated as shaded boxes. ALT, alanine aminotransferase; anti-HBS, hepatitis B surface antibody; COI, cutoff index; HBsAg, hepatitis B surface antigen; LDLT, living donor liver transplantation.

anti-HBs-escape mutations in HBsAg-positive recipients occurred 1–20 months after liver transplantation [22]. According to these results, the regular evaluation of HBV should be initiated just after liver transplantation and continued for the patient's lifetime.

The natural clinical course after *de novo* activation of HBV without nucleoside analogue treatment has been revealed in previous reports. We reported on a total of 19 cases of *de novo* activation of HBV after LDLT [7,13] without treatment after HBV activation. Overall, 16 of the 19 (84%) recipients, including 1 patient with anti-HBs escape mutant HBV, became HBsAg carriers; 1 died of fibrosing cholestatic hepatitis and only 2 patients spontaneously resolved to an HBsAg-negative state. Similar results showing that a majority of patients suffering from *de novo* activation of HBV after liver transplantation developed liver cirrhosis or chronic hepatitis have been reported [5,6,27]. These results indicate that most patients with *de novo* activation of HBV after liver transplantation enter an HBsAg carrier state without anti-HBV treatment because of the immunosuppressive conditions. Therefore, an effective management strategy is required for patients with *de novo* activation of HBV after liver transplantation.

We recently reported the beneficial effects of short-term lamivudine treatment for *de novo* activation of HBV caused by reasons other than anti-HBs escape mutations [13]. Lamivudine administration during the acute phase of *de novo* activation of HBV resulted in complete clearance of HBsAg from the serum in five of six patients, and all five remained negative for HBsAg even after the termination of lamivudine treatment. However, as shown in the present study, clearance of

Table 3. Treatment for seven patients with *de novo* activation of HBV with anti-HBs escape mutation after liver transplantation

Patient number	Treatment	Present status	Follow-up period after HBV activation, months	Duration from initiation of NA to disappearance of HBsAg, months	Duration of NA treatment, months	Present treatment
1	Entecavir	Resolved	28.0	2.5	5.8	None
2	Entecavir	Resolved	30.6	3.3	5.9	None
3	Entecavir	Resolved	30.5	6	9.9	None
4	Entecavir	Resolved	18.9	2.5	18.9	Entecavir
5	Lamivudine plus adefovir	Chronic hepatitis	59.8	-	59.8	Lamivudine plus adefovir
6	Lamivudine plus adefovir	Resolved	67.8	14.1	67.8	Adefovir
7	None	Chronic hepatitis	125.1	-	-	None

Anti-HBs, hepatitis B surface antibody; HBsAg, hepatitis B surface antigen; NA, nucleoside analogue.

HBsAg was not achieved in two patients with HBV with anti-HBs escape mutation by lamivudine administration. Although one patient achieved clearance of HBsAg after administration of adefovir, another patient developed chronic hepatitis B despite adding adefovir. In contrast, we demonstrated here the potent efficacy of entecavir on HBV with anti-HBs-escape mutations. The reason for the difference in efficacy between lamivudine and entecavir is unclear. Recent reports indicate that the overlap of the gene encoding HBsAg by the polymerase gene creates a unique situation in which a change within the polymerase gene following nucleoside analogue treatment might result in structural changes in the HBsAg protein and a subsequent reduction in the antigenicity of the protein. Lamivudine-resistant mutations in the polymerase gene are, indeed, associated with changes in the HBsAg protein, with a consequent reduction in antigenicity of the HBsAg protein comparable to that of anti-HBs-escape mutants [28,29]. The reverse might also be true. It has been reported that anti-HBs-escape mutations can produce a functionally significant alteration in the viral polymerase and influence the viral replication phenotype [30]. Both entecavir and lamivudine are nucleoside analogues that inhibit the HBV polymerase, but the mechanism of inhibition is different between these two nucleoside analogues. Entecavir inhibits HBV replication at three different steps: the priming of HBV DNA polymerase, reverse transcription of the negative-strand HBV DNA from the pregenomic RNA, and synthesis of the positive-strand HBV DNA, whereas lamivudine lacks the effect of the priming of HBV DNA polymerase [31]. The difference in the mechanism of HBV polymerase inhibition between entecavir and lamivudine may contribute to the difference in the efficacy of each nucleoside analogue on HBV with anti-HBs-escape mutations.

In conclusion, escape mutations from anti-HBs caused *de novo* activation of HBV under HBIG prophylaxis after liver transplantation from donors with resolved hepatitis B. Early administration of entecavir is important to avoid the subsequent development of acute liver failure or chronic hepatitis caused by *de novo* activation of HBV with anti-HBs-escape mutants.

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Disclosure statement

The authors declare no competing interests.

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The Pattern-Recognition Receptor NOD1 Promotes Production of Inflammatory Mediators in Rheumatoid Arthritis Synovial Fibroblasts

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ABSTRACT

Objective: Previously, we reported that pattern-recognition receptors (PRRs) such as TLRs and NOD2 contribute to the pathogenesis of rheumatoid arthritis (RA). Now, we analyzed the expression, regulation and function of the PRR NOD1 in RA synovial fibroblasts (RASFs) and its interaction with other PRRs.

Methods: Expression of NOD1 was analyzed by immunohistochemistry in RA, psoriasis arthritis, gout and OA synovial tissues. RASFs and monocyte-derived macrophages (MDMs) were stimulated with Tri-DAP, Pam3, PIC, LPS, heat inactivated bacteria, TNF-α or IL-1β. IL-6, CCL5, MMPs, NODs and TLRs were measured by Real-time PCR and/or ELISA. NOD1 and NOD2 were silenced with target specific siRNA. Phosphorylation of IRAK1 was measured by Western blot.

Results: In RA synovium, expression of NOD1 protein was significantly increased compared to OA synovium. There was similar basal expression of NOD1 in RASFs, OASFs, healthy controls PBMCs and MDMs. TLR3 stimulation further up-regulated NOD1 expression in RASFs. Expression of IL-6, CCL5, MMPs, TLR2 and NOD2 was significantly up-regulated in RASFs by stimulation with the NOD1 ligand. There was a synergistic effect in IL-6 production by stimulation with NOD1 and TLR2 ligands and NOD1 and TLR4 ligands. Silencing of NOD1, but not NOD2 decreased IL-6 levels after TLR2 and IL-1β stimulation and blocked phosphorylation of IRAK1.

Conclusion: NOD1 is strongly expressed in synovial tissues from RA patients in different cell types. Our data indicate that NOD1 alone and in interaction with other inflammatory activators plays an important role in the chronic and destructive joint inflammation in RA.