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REVIEW

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

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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.

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.

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.

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.

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.

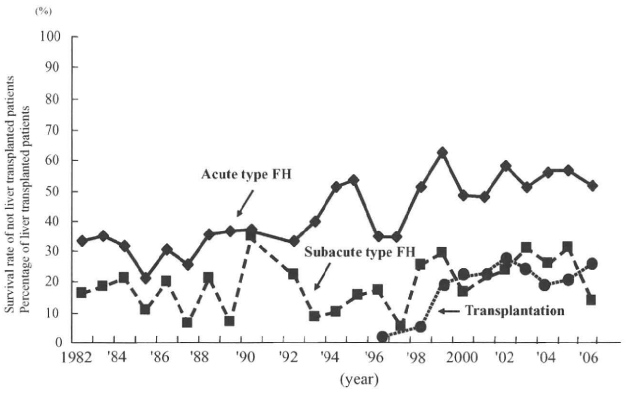

Figure 1 Survival rate of not liver transplanted patients with fulminant hepatitis(FH) and percentage of liver transplanted patients.

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.

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.

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

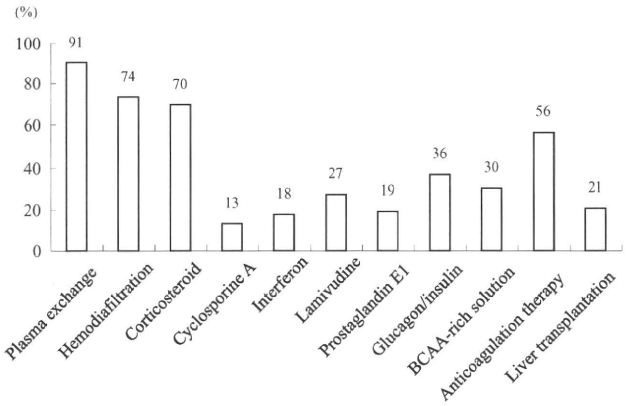

Figure 2 Percentage incidence of therapies performed for fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006). BCAA, branched-chain amino acid.

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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References

1 Trey C, Davidson CS. The management of fulminant hepatic failure. In: *Progress in Liver Failure*. New York: Grune and Stratton, 1970; 292–8.
2 Gimson AE, O'Grady J, Ede RJ, Portmann B, Williams R. Late onset hepatic failure: clinical, serological and histological features. *Hepatology* 1986; **6**: 288–94.
3 Bernuau J, Rueff B, Benhamou JP. Fulminant and subfulminant liver failure: definitions and causes. *Semin. Liver Dis.* 1986; **6**: 97–106.
4 O'Grady JG, Schalm SW, Williams R. Acute liver failure: redefining the syndromes. *Lancet* 1993; **342**: 273–5.
5 The Proceedings of the 12th Inuyama Symposium. *Hepatitis Type A and Fulminant Hepatitis*. Tokyo: Chugai Igaku-Sha, 1982; (In Japanese.).
6 Mochida S, Fujiwara K. Symposium on clinical aspects in hepatitis virus infection. Recent advances in acute and fulminant hepatitis in Japan. *Intern. Med.* 2001; **40**: 175–7.
7 Takahashi Y. Acute hepatic failure—in special relation to treatment. *Jpn. J. Med.* 1983; **22**: 140–5.
8 Fujiwara K, Mochida S, Matsui A. Fulminant hepatitis and LOHF in Japan. Annual Report of Intractable Liver Diseases Study Group of Japan, the Ministry of Health, Welfare and Labor (2001). 2002; 62–77. (In Japanese).
9 Fujiwara K, Mochida S, Matsui A, Nakayama N, Nagoshi S, Toda G; Intractable Liver Diseases Study Group of Japan. Fulminant hepatitis and late onset hepatic failure in Japan. *Hepatol. Res.* 2008; **38**: 646–57.
10 Mori M, Itanai F, Washio S *et al.* Estimated number of patients with intractable liver diseases in Japan based on nationwide epidemiology surveillance. Annual Report of Epidemiology Research for Intractable Diseases in Japan, the Ministry of Health, Welfare and Labor (2005). 2006; 39–42. (In Japanese).
11 Suzuki K, Takikawa Y, Endo T. Prospective study to clarify the prognosis of patients with fulminant hepatitis and acute hepatitis severe type. Annual Report of Intractable Liver Diseases Study Group of Japan, the Ministry of Health, Welfare and Labor (2001).2002; 87–96. (In Japanese).
12 Sato S, Suzuki K, Takikawa Y *et al.* Japanese National Study Group of Fulminant Hepatitis. Clinical epidemiology of fulminant hepatitis in Japan before the substantial introduction of liver transplantation: an analysis of 1309 cases in a 15-year national survey. *Hepatol. Res.* 2004; **30**: 155–61.
13 Yano K, Tamada Y, Yatsuhashi H *et al.* Japan National Hospital Acute Hepatitis Study Group. Dynamic epidemiology of acute viral hepatitis in Japan. *Intervirology* 2010; **53**: 70–5.
14 Kiyohara T, Sato T, Totsuka A, Miyamura T, Ito T, Yoneyama T. Shifting seroepidemiology of hepatitis A in Japan, 1973–2003. *Microbiol. Immunol.* 2007; **51**: 185–91.
15 Rezende G, Roque-Afonso AM, Samuel D *et al.* Viral and clinical factors associated with the fulminant course of hepatitis A infection. *Hepatology* 2003; **38**: 613–8.
16 Eto T, Shiraki K. National project on the prevention of mother-to-infant infection by hepatitis B virus in Japan. *Acta Paediatr. Jpn.* 1989; **31**: 681–4.
17 Hui CK, Cheung WW, Zhang HY *et al.* Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. *Gastroenterology* 2006; **131**: 59–68.
18 Yeo W, Chan TC, Leung NW *et al.* Hepatitis B virus reactivation in lymphoma patients with prior resolved hepatitis B undergoing anticancer therapy with or without rituximab. *J. Clin. Oncol.* 2009; **27**: 605–11.

19 Umemura T, Tanaka E, Kiyosawa K *et al.* Mortality secondary to fulminant hepatic failure in patients with prior resolution of hepatitis B virus infection in Japan. *Clin. Infect. Dis.* 2008; **47**: e52–6.
20 Kusumoto S, Tanaka Y, Mizokami M, Ueda R. Reactivation of hepatitis B virus following systemic chemotherapy for malignant lymphoma. *Int. J. Hematol.* 2009; **90**: 13–23.
21 Pitini V, Sturniolo G, Arrigo C, Leonardi S, Pino S, Altavilla G. HCV genotype 2 as a risk factor for reactivation in patients with B-cell lymphoma undergoing rituximab combination chemotherapy. *Br. J. Haematol.* 2010; **150**: 116–8.
22 Aggarwal R, Naik S. Epidemiology of hepatitis E: current status. *J. Gastroenterol. Hepatol.* 2009; **24**: 1484–93.
23 Suzuki K, Aikawa T, Okamoto H. Fulminant hepatitis E in Japan. *N. Engl. J. Med.* 2002; **347**: 1456.
24 Yazaki Y, Mizuo H, Takahashi M *et al.* Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. *J. Gen. Virol.* 2003; **84**: 2351–7.
25 Tei S, Kitajima N, Takahashi K, Mishiro S. Zoonotic transmission of hepatitis E virus from deer to human beings. *Lancet* 2003; **362**: 371–3.
26 Inoue J, Ueno Y, Nagasaki F *et al.* Sporadic acute hepatitis E occurred constantly during the last decade in northeast Japan. *J. Gastroenterol.* 2009; **44**: 329–37.
27 Ferrari R, Pappas G, Agostinelli D *et al.* Type 1 autoimmune hepatitis: patterns of clinical presentation and differential diagnosis of the "acute" type. *QJM* 2004; **97**: 407–12.
28 Abe M, Hiasa Y, Masumoto T *et al.* Clinical characteristics of autoimmune hepatitis with histological features of acute hepatitis. *Hepatol. Res.* 2001; **21**: 213–9.
29 Uetrecht J. Idiosyncratic drug reactions: past, present, and future. *Chem. Res. Toxicol.* 2008; **21**: 84–92.
30 Lammert C, Bjornsson E, Niklasson A, Chalasani N. Oral medications with significant hepatic metabolism at higher risk for hepatic adverse events. *Hepatology* 2010; **51**: 615–20.
31 Miyake Y, Iwasaki Y, Takaki A *et al.* Lamivudine treatment improves the prognosis of fulminant hepatitis B. *Intern. Med.* 2008; **47**: 1293–9.
32 Degertekin B, Lok AS. Indications for therapy in hepatitis B. *Hepatology* 2009; **49** (Suppl. 5): S129–37.
33 Jochum C, Gieseler RK, Gawlista I *et al.* Hepatitis B-associated acute liver failure: immediate treatment with entecavir inhibits hepatitis B virus replication and potentially its sequelae. *Digestion* 2009; **80**: 235–40.
34 Harry R, Auzinger G, Wendon J. The effects of supraphysiological doses of corticosteroids in hypotensive liver failure. *Liver Int.* 2003; **23**: 71–7.
35 Ichai P, Duclos-Vallée JC, Guettier C *et al.* Usefulness of corticosteroids for the treatment of severe and fulminant forms of autoimmune hepatitis. *Liver Transpl.* 2007; **13**: 996–1003.
36 Yokoi T, Oda S, Shiga H *et al.* Efficacy of high-flow dialysate continuous hemodiafiltration in the treatment of fulminant hepatic failure. *Transfus. Apher. Sci.* 2009; **40**: 61–70.
37 Inoue K, Kourin A, Watanabe T *et al.* Artificial liver support system using large buffer volumes removes significant glutamine and is an ideal bridge to liver transplantation. *Transplant. Proc.* 2009; **41**: 259–61.
38 Matsui Y, Sugawara Y, Yamashiki N *et al.* Living donor liver transplantation for fulminant hepatic failure. *Hepatol. Res.* 2008; **38**: 987–96.
39 Mochida S, Nakayama N, Matsui A *et al.* Re-evaluation of the Guideline published by the Acute Liver Failure Study Group of Japan in 1996 to determine the indications of liver transplantation in patients with fulminant hepatitis. *Hepatol. Res.* 2008; **38**: 970–9.
40 Mochida S, Naiki T, Takikawa Y *et al.* Fulminant hepatitis and LOHF in Japan. Revision of the guideline for liver transplantation Annual Report of Intractable Hepato-biliary Diseases Study Group of Japan, the Ministry of Health, Welfare and Labor (2001). 2009; 16–8. (In Japanese).
41 Ido A, Tsubouchi H. Translational research to identify clinical applications of hepatocyte growth factor. *Hepatol. Res.* 2009; **39**: 739–47.
42 Terai S, Sakaida I. Current status of autologous bone marrow cell infusion therapy for liver cirrhosis patients. *Hepatol. Res.* 2008; **38** (suppl. 1): S72–5.

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6. E型肝炎ウイルスの感染培養系

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これまでに，E型肝炎ウイルス(HEV)の初代肝細胞や種々の株化細胞での培養が試みられてきたが，いずれも増殖レベルは極めて低かった．最近，著者らは高力価のHEV(JE03-1760F株[遺伝子型3型]，あるいはHE-JF5/15F株[4型])を含む糞便浮遊液を用い，肝癌細胞株PLC/ PRF/5と肺癌細胞株A549での効率的なHEVの培養系を確立することに成功した．培養上清中のHEV RNA量は10^8 copies/mlに達し，新たな細胞への継代培養も可能であった．野生株JE03-1760Fと同等の増殖能を持つ感染性cDNAクローンを作製することができ，ORF3欠損変異クローンを用いて解析した結果，ORF3蛋白は感染細胞からのHEV粒子の放出に重要な役割を果たしていることが明らかになった．さらに，E型肝炎患者に由来する急性期血清中のHEVも，ウイルス量が多いほど，効率よくPLC/PRF/5細胞やA549細胞に感染し増殖しうることが分かった．効率的なHEVの感染培養系が確立されたことで，これまで未解明であったHEVに関する多方面の数々の疑問に一つ一つ答えを出すことが可能になったと言える．

はじめに

E型肝炎ウイルス（HEV）は急性あるいは劇症E型肝炎の原因ウイルスである．HEVはエンベロープに覆われていない小型球状粒子（直径27～34 nm：平均30 nm）であり，ヘペウイルス科（family *Hepeviridae*）のヘペウイルス属（genus hepevirus）に分類されている[1]．ゲノムは5'末端にキャップ構造，3'末端にポリA配列をもつ，約7,200塩基長の1本鎖（プラス鎖）RNAであり（**図1**），3つのopen reading frame（ORFs：ORF1，ORF2，ORF3）を有する[2]．ORF1はhelicaseやRNA polymeraseなどの非構造蛋白を，ORF2はcapsid蛋白をコードしている．ORF3は113ないし114アミノ酸残基からなる短いリン酸化蛋白をコードしている．ORF1蛋白はゲノムRNAから，ORF2蛋白とORF3蛋白は2.2-kbのサブゲノムRNAから翻訳される[3,4]．

HEVは主として肝臓で増殖し，胆管を経由して腸管に排出され，糞便とともに体外に放出される．そのため，不備な衛生環境下では糞口（fecal-oral）ルートによるHEV感染が起こり易く，アジア，アフリカおよび中米の熱帯・亜熱帯地域に位置する発展途上国は高い浸淫率を示している．一方，従前，日米欧等の先進諸国でのE型肝炎は，稀な「輸入感染症」の一つと理解され，殆ど注目されていなかった．ところが，1990年代末になって，流行地への渡航歴がない欧米の急性肝炎患者から新種のHEVが発見され，先進国には輸入感染症としてのE型肝炎のみならず，国内感染型のE型肝炎も存在することが次第に認識されるようになった．わが国でも2001年以降，海外渡航によらない散発性E型肝炎症例の存在が明らかになり[5-7]，国内の養豚場のブタでHEV感染が蔓延している事実が周知になった[8,9]．加えて，ブタや野生動物の肉・内臓摂食後のE型肝炎発症事例が相次いで報告され[10,11]，かつ重症化例や劇症肝炎による死亡例の存在も認識されるようになって[12]，E型肝炎がにわかに注目を集め，診断や疫学に関する研究も急速に進展した．その結果，本症における動物由来感染（zoonosis）の重要性が，わが国において，世界に先駆けて認知された．HEVの血清型は1種類であるが，1型から4型までの4種類の遺伝子型に大別されている[13]．それらは全塩基配列が互いに約25%異なっている．1型と2型がヒトのみに感染

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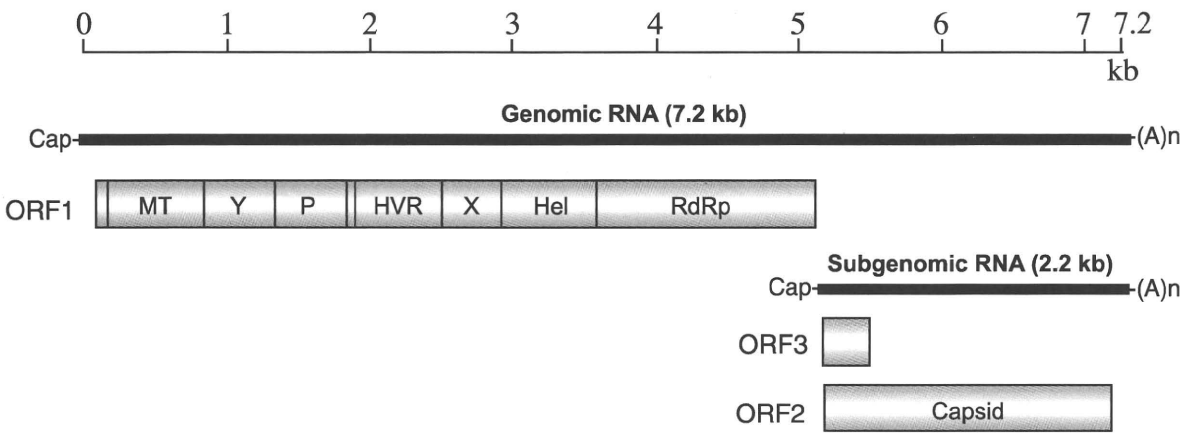

図1 HEVの遺伝子構造

MT, methyltransferase; Y, Y domain; P, papain-like protease; HVR, hypervariable region; X, X domain; Hel, helicase; RdRp, RNA-dependent RNA polymerase

表1 HEV遺伝子型とE型肝炎の特徴

遺伝子型	主な分布地域	主な感染様式	発展途上国	先進国	特徴
1	アジア アフリカ	水系感染(流行性)	主	稀(輸入感染)	● 若年成人に多い ● 妊婦での重症化
2	メキシコ アフリカ*				
3	アフリカを除く世界各地	食感染(散発性)	稀	主	● 人獣共通感染症(ブタやイノシシ、シカなどにも感染) ● 中高年男性に多い ● 重症化・劇症化例は中高年男性 ● 臓器移植患者では慢性化もある
4	中国、台湾、日本(北海道) ベトナム、インド、インドネシア				

*エジプト、チャド、ナミビア、ナイジェリア、中央アフリカ共和国、コンゴ民主共和国など。

し浸淫地域での流行性肝炎に関係しているのに対して，3型と4型はヒトのみならずブタやイノシシなどの動物にも感染し，人獣共通感染症としての散発性E型肝炎の原因となっている（**表1**）[13]．

つい最近まで，HEVの培養系が無いことがウイルス学的研究を展開するうえでの大きな障壁となっていたが，著者らはHEVの効率的な感染培養系を確立することに成功し，これまでに未解明であったウイルス本態の解析が急速に進展しつつある．本稿ではHEVの感染培養系の確立およびそれによって得られた新しい知見を紹介したい．

1. HEVの感染培養系の確立

5種類の既知の肝炎ウイルスのうち，A型肝炎ウイルス(HAV)については，1979年にProvostら[14]によって培養系が確立され，国内でも不活化HAワクチンの製造に応用されている．また，C型肝炎ウイルス(HCV)については，2005年に脇田ら[15]によって劇症C型肝炎患者から分離された遺伝子型2aのJFH-1ウイルスを用いたin vitro系が樹立され，HCVの感染・複製・粒子形成機構の解析に大きな進展が見られている．しかし，HEVの培養系については1990年代初頭から多くの研究者によって試みられてきたが[16-22]，増殖効率が極めて低く，継代培養も困難であり，HEVの物理化学的性状の解析やウイルス学的研究に供することは出来なかった．

2003年に1人の国内感染のE型肝炎患者から高力価のHEV（JE03-1760F株[遺伝子型3型]：2.0×10^7 copies/ml）を含む糞便サンプルが得られたことが，これから述べるHEVの培養系樹立の契機となった．その患者に由来する糞便浮遊液を接種材料として，ヒトやサル，ウシ，イヌ，ラット，マウスに由来する21種類の株化細胞株でそのHEV株が増殖可能かどうかを検討した結果，肝癌細胞株PLC/ PRF/5（Alexander）と肺癌細胞株A549の2種類の細胞株でJE03-1760F株が効率よく増殖しうることが分かった[23]．接種ウイルス量に依存して効率よく培養上清中に

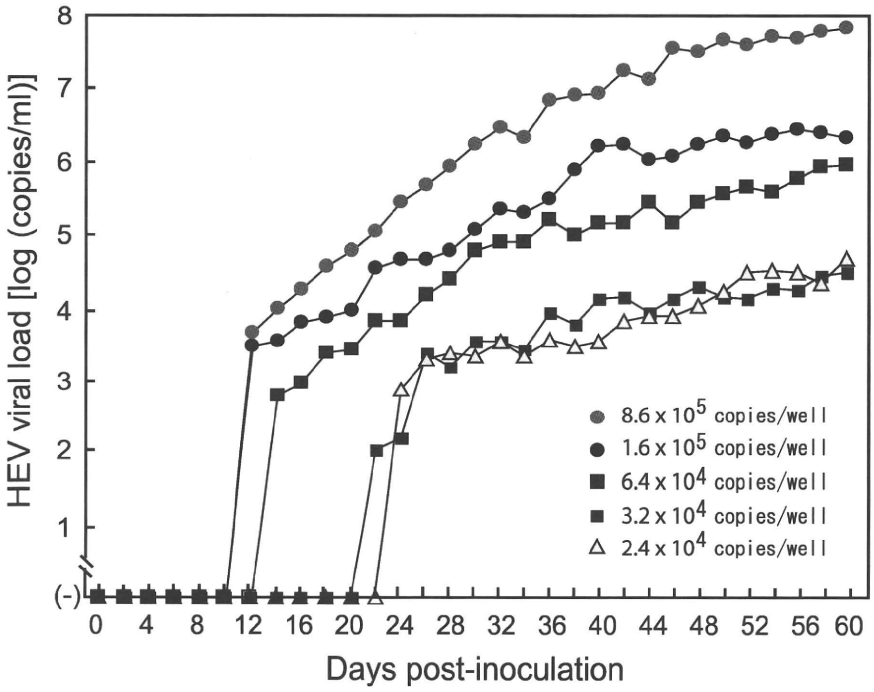

図 2　肝癌細胞株 PLC/PRF/5 における HEV の増殖
種々のウイルス量の HEV(JE03-1760F 株)を含む糞便浮遊液を接種したあとの 60 日目までの培養上清中の HEV RNA titer を示す.

子ウイルスが産出され，6-well plate の 1 well 当たりの copy 数を 8.6 x 10^5（MOI ≒ 0.9）として JE03-1760F 株を接種したところ，60 日目には培養上清中のウイルス量が 10^8 copies/ml に達した(**図 2**). また, 1 well 当たりの copy 数を 2.4 x 10^4（MOI=0.03）に減らしても増殖を確認することができた．さらに，培養上清中に放出された子ウイルスは新たな PLC/PRF/5 細胞や A549 細胞でも効率良く増え，かつ連続的な継代培養も可能であることが分かった．高いウイルス量の接種材料が得られたこと，そしてその HEV 株が既知の 3 型 HEV 株には認められないユニークな変異を 29 ヶ所の塩基（そのうち，6 塩基はアミノ酸置換を伴う非同義変異）を有していたことが，世界で初めての効率的な感染培養系の確立に繋がったものと推測される[24].

続いて，別の E 型肝炎患者（劇症型）から得られた高力価の HEV（HE-JF5/15F 株[遺伝子型 4 型]：1.3 x 10^7 copies/ml）を含む糞便浮遊液を接種することにより，新たに 4 型 HEV 株の培養系を確立することができた[25]. JE03-1760F 株と同様に，培養上清中の HEV を用いた継代培養が可能であるだけでなく，6 代目の継代培養において，接種後 2 日目に培養上清中で子ウイルス（HE-JF5/15F_p6）の産出が確認され，ウイルスタイターは 10 日目に 1.5 x 10^8 copies /ml に達した（**図 3**）．疫学データから，遺伝子型 4 と肝炎重症化との密接な関連性が示唆されているが[26]，劇症肝炎患者から分離された 4 型 HEV 株の活発な増殖能が本培養系で再現されたことは劇症肝炎発症のウイルス因子を解明するうえで興味深い.

2. 細胞培養に伴う HEV の馴化と遺伝子変異

培養細胞への HEV の馴化にどのような遺伝子変異が関わっているかを明らかにすることを目的として，野生株である 糞便中の JE03-1760F 株を出発材料として，2 系列（実験 A と実験 B）の継代実験を行った[27]．実験 A では，接種ウイルス量を約 10^5 copies/well として Passage 10 まで継代実験を行ったところ，前半の Passages 0-5 に比べて後半の Passages 6-10 の方が，接種後ウイルスが出現するまでの平均日数が約 1 週間短縮され（16.7 日 vs. 10.0 日），平均で 19 日も早く培養上清中のウイルス量が 10^5 copies/ml に達した(35.2 日 vs. 16.0 日). また, Passage 11, Passage 12, Passage 13 の継代実験では，接種ウイルス量をそれぞれ 3.0 x 10^3 copies/well, 1.0 x 10^3 copies/well, 3.0 x 10^2 copies/well として行い，より増殖効率の高い株を順次選別することができた．その結果，Passage 13 で得られた継代株（p13/A）は野生株の 30 分の 1 のウイルス量で感染しうることが分かった．実験 B においても実験 A 同様，前半の Passages 0-5 に比べて，後半の Passages 6-10 の方がウイルス出現までの平均日数が短くなり，2 日目に検出されるようになり，培養上清中のウイルス量が 10^5 copies/ml に達するまでの平均日数が約 1 週間短縮され，接種後 10 日目には 10^5 copies/ml を超えるようになった[27].

実験 A での Passage 13 の株（p13/A 株）と実験 B での

表2 野生株 JE03-1760F と2系列の継代株(p13/A と p10/B)の全塩基配列の比較

Nt no.	Region	Nucleotides			Amino acids	
		Wild-type	p13/A	p10/B	Residue no.	Mutation
61	ORF1	U	U	C	12	-
370	ORF1(MT)	C	U	C	115	-
445	ORF1(MT)	U	U	C	140	-
591	ORF1(MT)	C	U	C	189	Ala to Val
829	ORF1(Y)	C	C	U	268	-
1378	ORF1(P)	C	C	U	451	-
1549	ORF1(P)	U	U	C	508	-
2191	ORF1(HVR)	C	C	U	722	-
2236	ORF1(HVR)	C	C	U	737	-
2246	**ORF1(HVR)**	**U**	**C**	**C**	**741**	**Trp to Arg**
2704	ORF1(X)	U	C	U	893	-
2808	ORF1(X)	U	U	C	928	Val to Ala
2913	ORF1(Hel)	A	A	G	963	Glu to Gly
2915	ORF1(Hel)	G	G	U	964	Val to Leu
2938	ORF1(Hel)	C	U	C	971	-
3106	ORF1(Hel)	A	G	A	1027	-
3223	ORF1(Hel)	U	U	C	1066	-
3235	ORF1(Hel)	C	U	C	1070	-
3453	ORF1(Hel)	C	U	C	1143	Ala to Val
3475	ORF1(Hel)	C	C	U		
3496	ORF1(Hel)	C	U	C	1157	-
3553	ORF1(Hel)	C	U	C	1176	-
3620	ORF1(Hel)	U	C	U	1199	-
4015	ORF1(RdRp)	C	U	C	1330	-
4309	ORF1(RdRp)	C	C	U	1428	-
4462	ORF1(RdRp)	C	U	C	1479	-
5312	ORF2	U	U	C	47	-
	ORF3				51	Ile to Thr
5378	**ORF2**	**A**	**G**	**G**	69	-
	ORF3				**73**	**Asn to Ser**
5456	**ORF2**	**C**	**U**	**U**	95	-
	ORF3				**99**	**Pro to Leu**
6047	ORF2	U	U	C	292	
6470	ORF2	C	U	C	433	-
6578	ORF2	C	U	C	469	-
6611	ORF2	C	U	C	480	-
6626	ORF2	U	C	U	485	-
6651	ORF2	G	G	R	494	Val to Ala/Thr
6652	ORF2	U	U	C	494	Val to Ala/Thr
6855	ORF2	A	A	G	562	Asn to Asp
6944	ORF2	U	U	C	591	-
7186	3'UTR	C	C	U	NA	-

註：MT, methyltransferase; Y, Ydomain; P, papain-like protease; HVR, hypervariable region; X, X domain; Hel, helicase; RdRp, RNA-dependent RNA polymerase.

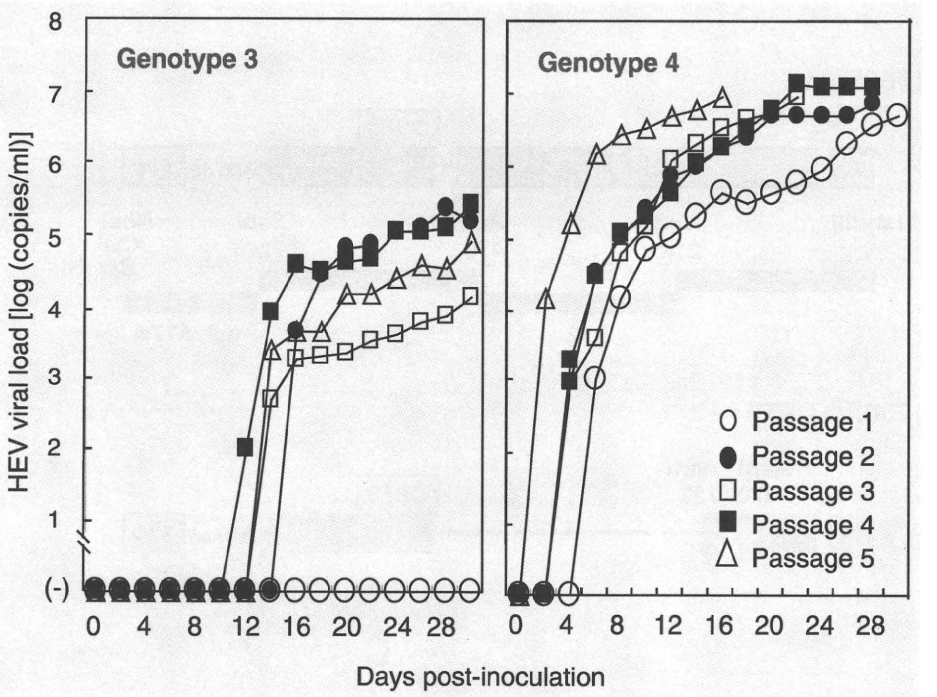

図 3　急性 E 型肝炎患者に由来する 3 型 HEV(JE03-1760F)と劇症 E 型肝炎患者に由来する 4 型 HEV(HE-JF5/15F)の肝癌細胞株 PLC/PRF/5 における増殖パターンの比較

培養上清中 HEV を接種したあとの，5 回の継代培養(Passages 1-5)における接種後 30 日目までの培養上清中の HEV RNA titer を示す．

Passage 10 の株（p10/B 株）について全塩基配列を決定したところ，野生株に比べて，7226 塩基（ポリ A 配列を除く）中，それぞれ 19 塩基と 23 塩基に相違が認められた（**表 2**）．p13/A 株に認められた 19 塩基の変異のうち，5 塩基が ORF1 あるいは ORF3 でのアミノ酸置換（それぞれ 3 個と 2 個）を伴っていた．また，p10/B 株に認められた 23 塩基の変異のうち，9 塩基が ORF1，ORF2 および ORF3 でのアミノ酸置換（それぞれ，4 個，3 個，2 個）を伴っていた．ウイルスゲノム全長の 0.3% に相当する様々な遺伝子変異が培養系への HEV の馴化に関連があることが示唆された．特に，2 系列の継代培養において p13/A 株と p10/B 株に共通に認められた変異（いずれもアミノ酸置換を伴う変異）は馴化に伴う HEV ゲノム変異の再現性を示すものであり，効率的な増殖能や感染性に密接な関連がある，特に重要な変異として注目される．

3. HEV の reverse genetics system の確立

HEV の感染性 cDNA クローンを作製するため，JE03-1760F 株のゲノム RNA を鋳型にして，RT-PCR 法によりゲノム全長をカバーする cDNA 断片を増幅し，その cDNA 断片を T7 プロモーターと poly（A）配列との間に挿入したゲノムプラスミドを構築した（**図 4A**）[28]．このゲノムプラスミドから in vitro transcription によりゲノム全長の RNA を合成し，5'末端にキャップを付加したのち，PLC/PRF/5 細胞に導入したところ，培養上清中に 10^7 copies/ml 以上の高いレベルでの HEV 産生が認められた（**図 4B**）．ΔORF1 は ORF1 に frameshift mutation を持った defective クローンであり，negative control として用いた．抗 ORF2 マウスモノクローナル抗体（mAb）（H6225）を用い，immunofluorescent assay（IFA）法により細胞内の ORF2 蛋白を検出した結果，ORF2 陽性細胞数はトランスフェクション後，5 日，7 日，11 日，15 日と日を追って増加し，HEV 感染が拡大している像が観察された（**図 4C**）．この cDNA 由来 HEV（pJE03-1760F/wt）は新たな PLC/PRF/5 細胞や A549 細胞に感染し効率よく増殖できるだけでなく，継代培養も可能であり，糞便由来野生株 JE03-1760F と同等の増殖能を持つことが明らかになった．

4. 変異 HEV クローンの作製と ORF3 蛋白の機能解析

HEV ORF3 蛋白の過剰発現系での実験結果から，このウイルス蛋白が細胞内で様々な機能を持った蛋白として存在していることは報告されているが[29]，生理的な条件下での機能は不明であり，粒子形成に与っているか否かについても分かっていなかった．そこで，ORF3 の ATG コドンを GCA に変異させた ORF3 欠損 cDNA クローン（ΔORF3）を作製し，その全長 RNA を PLC/PRF/5 細胞に transfect

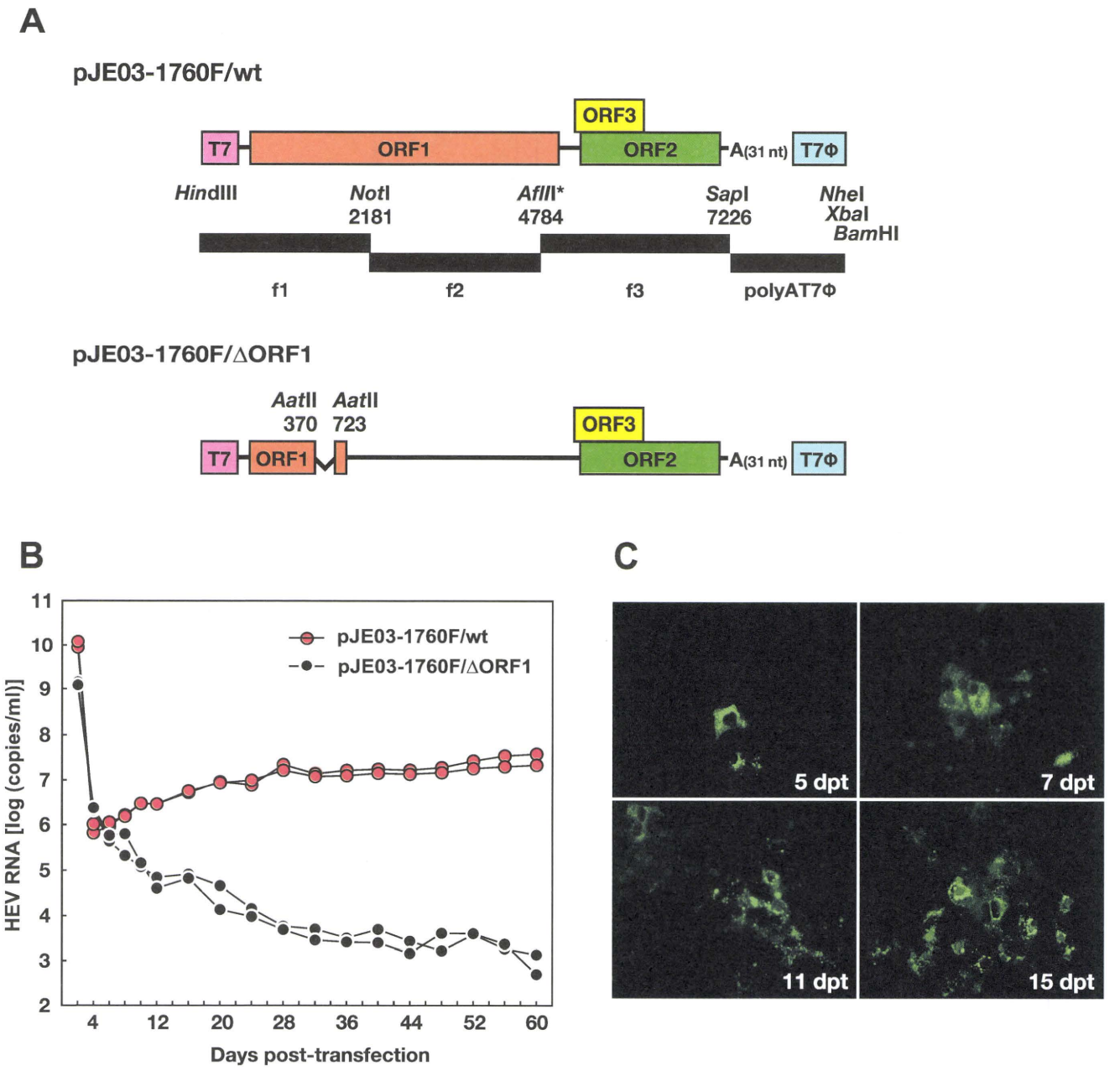

図4 HEVの感染性cDNAクローンの作製と評価

(A) 野生株JE03-1760FのcDNAクローンおよびnegative controlとしてのORF1欠損変異クローン(Δ ORF1)の構築

(B) 全長RNAをPLC/PRF/5細胞にtransfectしたあとの培養上清中のHEV RNA titerの推移を示す．野生株とΔ ORF1のそれぞれについて2 wellsのデータを示す．

(C) IFA: 抗ORF2 mAb (H6225)およびAlexa Fluor 488標識抗マウスIgGを用いて細胞内のORF2抗原を検出した．

した[30]．その結果，Δ ORF3ウイルスでは細胞内で野生株pJE03-1760F/wtと同等レベルのHEV RNAが検出されたにも拘わらず，培養上清中への子ウイルスの放出は認められなかった（図5）．したがって，ORF3欠損ウイルスは細胞内では増殖できても分泌能を欠いていることが明らかになった．

加えて，抗ORF3 mAb（TA0536）を用いたimmunocapture PCR法によって検討した結果，糞便中のHEV粒子と異なり，培養上清中のHEV粒子上にORF3蛋白が存在していることが明らかになった[30,31]．界面活性剤非存在下での捕捉率は数％に過ぎず，種々の界面活性剤による処理によって捕捉率は約100％に達することから，ORF3蛋白は培養上清中のHEV粒子上でリピドを含んだ膜にほぼ覆われた状態で存在しているものと考えられる．また，糞

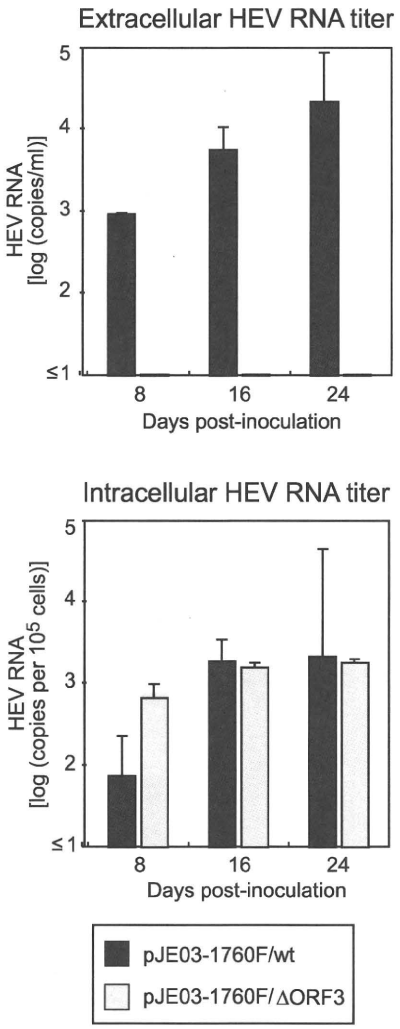

図 5　野生株と ORF3 欠損株(Δ ORF3)での培養上清中および細胞内の HEV RNA titer の比較

便中の HEV 粒子を抗 ORF3 mAb で捕捉できなかったのは，HEV 粒子が肝臓から胆管に放出され，腸管に排泄される過程で胆汁中のデオキシコール酸と膵液中の蛋白分解酵素（トリプシン）に曝され，細胞膜とともに ORF3 蛋白が除去されることに起因するものと考えられた．実際，培養上清中の HEV 粒子をデオキシコール酸とトリプシンで処理することにより，抗 ORF2 mAb によってほぼ完全に捕捉されるのに対して，抗 ORF3 mAb によっては全く捕捉されなくなり，ショ糖液中での浮上密度も処理前の 1.15-1.16 g/ml から糞便中 HEV 粒子と同等の 1.27-1.28 g/ml にシフトした．

したがって，ORF3 蛋白は粒子上に存在すると言う点で構造蛋白でもあり，感染細胞からの放出に重要な役割を果たしていると考えられる．そして，培養上清中の HEV が細胞膜に覆われ，ORF3 蛋白を担った状態で細胞から放出され，恰も “enveloped” ウイルスとして存在しているのに対して，糞便中ではこれまでの教科書に記載されているように “non-enveloped” ウイルスとして存在しており，HEV 粒子として 2 種類の存在形態があることが分かった．

5. 患者血清由来 HEV の感染培養系の確立

E 型肝炎患者血清中の HEV 粒子の浮上密度を測定すると，培養上清中の HEV 粒子と同じように，ショ糖液中で 1.15-1.16 g/ml にピークを形成する．培養上清中の HEV 粒子は糞便中の HEV 粒子と異なり，“enveloped” ウイルスの形態をとりながら，新たな培養細胞に感染し，効率よく増えうる．そして，輸血後 E 型肝炎の発生も国内外から報告がある[32,33]．このような事実から，血清中 HEV 粒子の感染培養系も確立できるのではないかと考え，以下のような接種実験を試みた．

輸入感染あるいは国内感染の E 型肝炎患者に由来する急性期血清（n=32）を 6-well plate の各 well 当たり 10^6 copies のオーダー（1.5-3.0 x 10^6 copies/well, n=4），あるいは 10^5 copies のオーダー（1.5-5.8 x 10^5 copies/well，n=9）

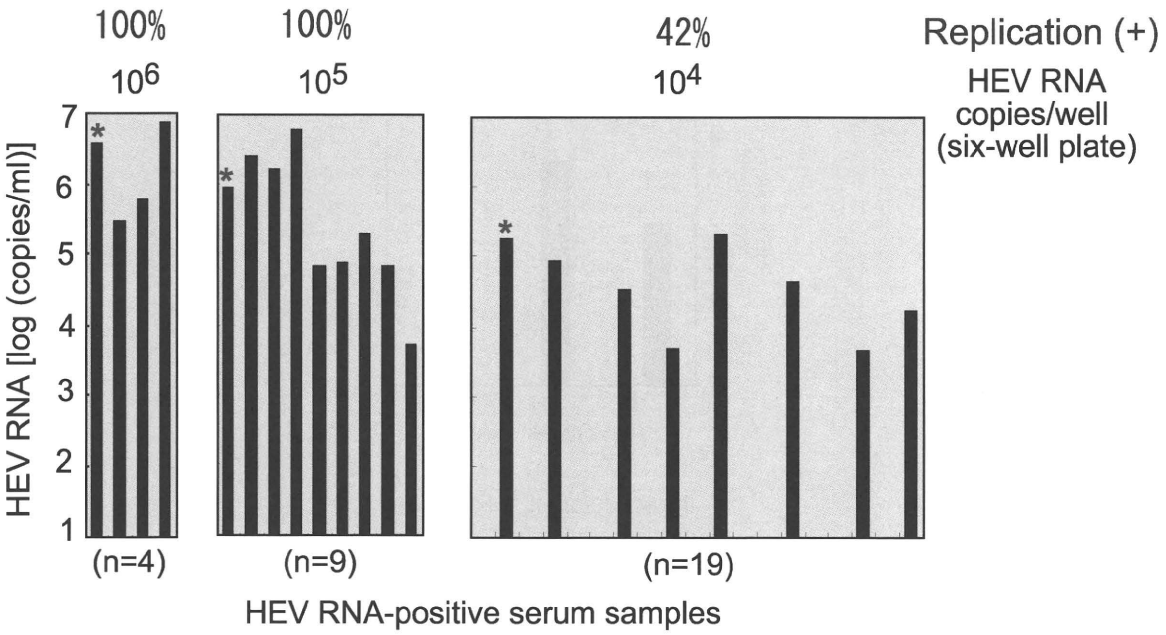

図6 血清中HEVをPLC/PRF/5細胞に接種したあとの30日目の培養上清中のHEV RNA titer
6-well plateの1well当たり10^6 copies，10^5 copies，あるいは10^4 copiesで血清中HEVを接種した場合の感染が認められた検体は，それぞれ100%(4/4)，100%(9/9)，42%(8/19)であった．*印はHEV抗体が陰性の検体であることを示す．

で接種したところ，全wellでHEVの増殖が認められ，接種後30日目の培養上清中のHEV RNA titerは10^6オーダーの接種では2.1 x 10^6 copies/ml（中央値），10^5オーダーの接種では1.9 x 10^5 copies/ml（中央値）に達し，接種ウイルス量が多いほど活発な増殖を示すことが分かった（**図6**）[34]．また，10^4オーダー（2.0-7.2 x 10^4 copies/well）の接種では19検体中8検体（42%）で増殖が観察され，それら8検体での接種後30日目の培養上清中のHEV RNA titerは3.9 x 10^4 copies/ml（中央値）であった（**図6**）．ORF2領域の412塩基長の配列を決定し，inoculumとして用いた血清中HEVと培養上清中HEV（30日目）を比較した結果，それぞれのサンプルについて，inoculumと100%一致するウイルスが培養細胞で産生されたことを確認できた．

また，培養上清中のHEVをA549細胞に接種したところ，どのHEV株も効率よく増殖することが観察され，培養上清中には感染性を有する子ウイルスが産出されていることが分かった[34]．

増殖が確認された21検体のうち，3検体（**図6**のなかで*印の付いた検体）のみ血清中HEV抗体が陰性であり，残りの18検体は感染性HEV粒子が存在しながらHEV抗体が共存していた．ショ糖液中での浮上密度を調べると，血清中のHEV粒子はHEV抗体の存否に関わらず，培養上清中のHEV粒子と同様，比重1.15-1.16 g/mlの分画にピークを形成した（**図7**）．また，HEV抗体が陰性の3検体，HEV抗体が陽性の4検体について，ヤギ抗ヒトIgG/IgM/IgA抗体を添加し，免疫沈降実験を行なった結果，予め回復期血清を加えてあった糞便中HEV粒子はほぼ100%沈殿物として回収されたのに対して，血清中HEV粒子はHEV抗体の共存にも関わらず，殆ど上清中で回収され，沈殿物中で回収されたHEV粒子はわずか8.1%以下であった．すなわち，血清中のHEV粒子の殆どはHEV抗体共存下でもimmune complexを形成せず，“free”の状態で存在していることが分かった[34]．

Tween 20やNP-40などの界面活性剤で予め処理を行なうことにより，血清中のHEV粒子は培養上清中のHEV粒子と同様に，抗ORF2抗体および抗ORF3抗体で部分的に捕捉されるようになったが，予め界面活性剤と蛋白分解酵素の両者を添加し処理を行なうと，血清中HEV粒子と培養上清中HEV粒子は糞便中HEV粒子と同様に，1.27-1.28 g/mlの浮上密度となり（**図7**），抗ORF2抗体によってほぼ100%捕捉され，抗ORF3抗体によっては全く捕捉されなくなった．界面活性剤や蛋白分解酵素による処理に対して血清中HEV粒子は培養上清中HEV粒子と同等の挙動を示すことが明らかになったことから，より高いウイルスタイターのサンプルが入手可能な培養上清中のHEV粒子を用い，処理後のHEV粒子の培養細胞への感染性を検討した．その結果，界面活性剤単独処理の粒子もさらに界面活性剤と蛋白分解酵素の処理を受けた粒子も未処理の粒子と同等の感染性を有することが分かった[34]．

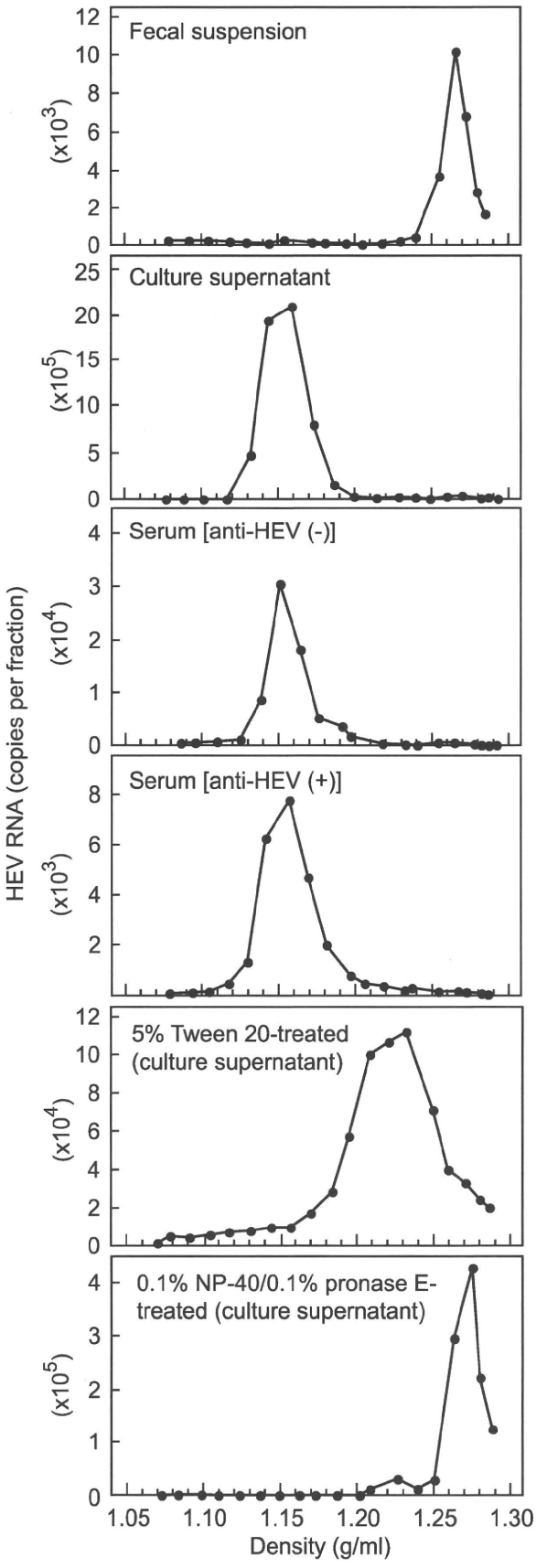

図 7　HEV 粒子のショ糖液中での浮上密度

本研究を通じて，E型肝炎患者に由来する急性期糞便中のHEV（JE03-1760F株[3型]とHE-JF5/15F株[4型]）のみならず，血清中のHEVもHEV抗体の有無に関わらず，ウイルスタイターが高いほど，効率よくPLC/PRF/5細胞やA549細胞に感染し増殖しうることが分かった．原血清中のHEV RNAタイターが概ね5.0 x 10^5 copies/ml以上であれば，これらの細胞内で効率よく増殖しうる．培養上清中に放出されたHEV粒子が細胞膜成分に覆われ，糞便中のHEV粒子と異なって浮上密度が軽いことは上述の通りであるが，血清中のHEV粒子も細胞膜成分に覆われ，“enveloped virus”様の粒子構造をとっており，HEV抗体が共存していても，その抗体が中和抗体として粒子に結合していないことが明らかになった．そのため，HEV抗体の存否は培養細胞への感染性には影響していないことが明らかになった．しかし，現時点では何故，糞便中のHEV粒子のような“non-enveloped”ウイルスと，血清中や培養上清中のHEV粒子のように“enveloped”ウイルス様の構造を持った粒子が同等の感染性を示しうるのかを明らかに出来ていない．これらの粒子がどのような機序で感受性細胞に吸着し侵入しうるのか，その解明はHEVの生活環を理解するうえで極めて重要であると思われる．

おわりに

本稿で紹介したような，培養上清中に高濃度のHEV粒子が産生分泌される培養系が確立されたのは，世界で初めてである．JE03-1760F株のみならず，他の複数の株でも同等，あるいはそれ以上の効率で増殖が可能性で，かつ連続的な継代培養を行うことができている．また，感染性cDNAクローンの構築にも成功している．これらの培養系およびreverse genetics systemを用いることにより，これまで未解明であったHEVに関する多方面の数々の疑問に一つ一つ答えを出すことが可能になったと言える．

さらに，PLC/PRF/5細胞やA549細胞を用いたHEVの感染培養系は，糞便中HEVのみならず，血清中の多くのstrainsの感染増殖を効率よくsupportしうることが分かった．このことで，本培養系の応用の幅が大きく広がったと言える．また，血清中のHEV粒子は培養上清中のHEV粒子と同様に，“enveloped”ウイルス様の粒子構造をとっていることが分かった．HAVについても，培養上清中の一部の粒子が細胞膜成分に覆われていることは知られているが[35,36]，HEVの場合は培養上清中および循環血液中の殆どすべての粒子が細胞膜成分に覆われている点でHAVと異なる．このように，血清中や培養上清中のHEV粒子が細胞膜成分に覆われていながら，何故，“non-enveloped”ウイルスの糞便中HEV粒子と同等の感染性を有するのかが分かっていない．そのmysteriousな現象の解明がHEVの感受性細胞への吸着・侵入のメカニズムを明らかにする上で重要である．

文 献

1) Emerson SU, Anderson D, Arankalle A, Meng XJ, Purdy M, Schlauder G.G, Tsarev, SA. (2004). *Hepevirus*. In *Virus Taxonomy*: VIIIth report of the ICTV, pp. 853-855. Edited by Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball, LA. Elsevier/Academic Press, London.
2) Tam AW, Smith MM, Guerra ME, Huang CC, Bradley DW, Fry KE, Reyes GR. Hepatitis E virus (HEV): molecular cloning and sequencing of the full-length viral genome. Virology 185:120-131, 1991.
3) Graff J, Torian U, Nguyen H, Emerson SU. A bicistronic subgenomic mRNA encodes both the ORF2 and ORF3 proteins of hepatitis E virus. J Virol 80:5919-5926, 2006.
4) Ichiyama K, Yamada K, Tanaka T, Nagashima S, Jirintai, Takahashi M, Okamoto H. Determination of the 5'-terminal sequence of subgenomic RNA of hepatitis E virus strains in cultured cells. Arch Virol 154:1945-1951, 2009.
5) Takahashi K, Iwata K, Watanabe N, Hatahara T, Ohta Y, Baba K, Mishiro S. Full-genome nucleotide sequence of a hepatitis E virus strain that may be indigenous to Japan. Virology 287:9-12, 2001.
6) Mizuo H, Suzuki K, Takikawa Y, Sugai Y, Tokita H, Akahane Y, Itoh K, Gotanda Y, Takahashi M, Nishizawa T, Okamoto H. Polyphyletic strains of hepatitis E virus are responsible for sporadic cases of acute hepatitis in Japan. J Clin Microbiol 40:3209-3218, 2002.
7) Okamoto H, Takahashi M, Nishizawa T. Features of hepatitis E virus infection in Japan. Intern Med 42:1065-1071, 2003.
8) Okamoto H, Takahashi M, Nishizawa T, Fukai K, Muramatsu U, Yoshikawa A. Analysis of the complete genome of indigenous swine hepatitis E virus isolated in Japan. Biochem Biophys Res Commun 289:929-936, 2001.
9) Takahashi M, Nishizawa T, Miyajima H, Gotanda Y, Iita T, Tsuda F, Okamoto H. Swine hepatitis E virus strains in Japan form four phylogenetic clusters comparable with those of Japanese isolates of human hepatitis E virus. J Gen Virol 84:851-862, 2003.
10) Tei S, Kitajima N, Takahashi K, Mishiro S. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet 362:371-373, 2003.
11) Yazaki Y, Mizuo H, Takahashi M, Nishizawa T, Sasaki N, Gotanda Y, Okamoto H. Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be foodborne, as suggested by the presence of hepatitis E virus in pig liver as food. J Gen Virol 84:2351-2357, 2003.
12) Suzuki K, Aikawa T, Okamoto H. Fulminant hepatitis E in Japan. N Engl J Med 347:1456, 2002.
13) Okamoto H. Genetic variability and evolution of hepatitis E virus. Virus Res 127:216-228, 2007.
14) Provost PJ, Hilleman MR. Propagation of human hepatitis A virus in cell culture in vitro. Proc Soc Exp Biol Med 160:213-21, 1979.

15) Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, Murthy K, Habermann A, Kräusslich HG, Mizokami M, Bartenschlager R, Liang TJ. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med 11:791-796, 2005.
16) Kazachkov Yu. A, Balayan MS, Ivannikova TA, Panina LI, Orlova TM, Zamyatina NA, Kusov Yu. Yu. Hepatitis E virus in cultivated cells. Arch Virol 127, 399-402, 1992.
17) Huang R, Nakazono N, Ishii K, Li D, Kawamata O, Kawaguchi R, Tsukada Y. I. Hepatitis E virus (87A strain) propagated in A549 cells. J Med Virol 47:299-302, 1995.
18) Tam AW, White R, Reed E, Short M, Zhang Y, Fuerst TR, Lanford RE. (1996). In Vitro propagation and production of hepatitis E virus from in vitro-infected primary macaque hepatocytes. Virology 215, 1-9, 1996.
19) Meng J, Dubreuil P, Pillot J. A new PCR-based seroneutralization assay in cell culture for diagnosis of hepatitis E. J Clin Microbiol 35:1373-1377, 1997.
20) Huang R, Li D, Wei S, Li Q, Yuan X, Geng L, Li X, Liu M. Cell culture of sporadic hepatitis E virus in China. Clin Diagn Lab Immunol 6, 729-733, 1999.
21) Wei T, Walsh P, Huang R, To SST. 93G, a novel sporadic strain of hepatitis E virus in south China isolated by cell culture. J Med Viol 61:311-318, 2000.
22) Emerson SU, Clemente-Casares P, Moiduddin N, Arankalle VA, Torian U, Purcell RH. Putative neutralization epitopes and broad cross-genotype neutralization of Hepatitis E virus confirmed by a quantitative cell-culture assay. J Gen Virol 87:697-704, 2006.
23) Tanaka T, Takahashi M, Kusano E, Okamoto H. Development and evaluation of an efficient cell-culture system for Hepatitis E virus. J Gen Virol 88:903-911, 2007.
24) Takahashi M, Tanaka T, Azuma M, Kusano E, Aikawa T, Shibayama T, Yazaki Y, Mizuo H, Inoue J, Okamoto H. Prolonged fecal shedding of hepatitis E virus (HEV) during sporadic acute hepatitis E: evaluation of infectivity of HEV in fecal specimens in a cell culture system. J Clin Microbiol 45:3671-3679, 2007.
25) Tanaka T, Takahashi M, Takahashi H, Ichiyama K, Hoshino Y, Nagashima S, Mizuo H, Okamoto H. Development and characterization of a genotype 4 hepatitis E virus cell culture system using a HE-JF5/15F strain recovered from a fulminant hepatitis patient. J Clin Microbiol 47:1906-1910, 2009.
26) Mizuo H, Yazaki Y, Sugawara K, Tsuda F, Takahashi M, Nishizawa T, Okamoto H. Possible risk factors for the transmission of hepatitis E virus and for the severe form of hepatitis E acquired locally in Hokkaido, Japan. J Med Virol 76:341-349, 2005.
27) Lorenzo FR, Tanaka T, Takahashi H, Ichiyama K, Hoshino Y, Yamada K, Inoue J, Takahashi M, Okamoto H. Mutational events during the primary propagation and consecutive passages of hepatitis E virus strain JE03-1760F in cell culture. Virus Res 137:86-96, 2008.
28) Yamada K, Takahashi M, Hoshino Y, Takahashi H, Ichiyama K, Tanaka T, Okamoto H. Construction of an infectious cDNA clone of hepatitis E virus strain JE03-1760F that can propagate efficiently in cultured cells. J Gen Virol 90:457-462, 2009.
29) Chandra V, Taneja S, Kalia M, Jameel S. Molecular biology and pathogenesis of hepatitis E virus. J Biosci 33:451-464, 2008.
30) Yamada K, Takahashi M, Hoshino Y, Takahashi H, Ichiyama K, Nagashima S, Tanaka T, Okamoto H. ORF3 protein of hepatitis E virus is essential for virion release from infected cells. J Gen Virol 90:1880-1891, 2009.
31) Takahashi M, Yamada K, Hoshino Y, Takahashi H, Ichiyama K, Tanaka T, Okamoto H. Monoclonal antibodies raised against the ORF3 protein of hepatitis E virus (HEV) can capture HEV particles in culture supernatant and serum but not those in feces. Arch Virol 153:1703-1713, 2008.
32) Matsubayashi K, Nagaoka Y, Sakata H, Sato S, Fukai K, Kato T, Takahashi K, Mishiro S, Imai M, Takeda N, Ikeda H. Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. Transfusion 44:934-940, 2004.
33) Mitsui T, Tsukamoto Y, Yamazaki C, Masuko K, Tsuda F, Takahashi M, Nishizawa T, Okamoto H. Prevalence of hepatitis E virus infection among hemodialysis patients in Japan: evidence for infection with a genotype 3 HEV by blood transfusion. J Med Virol 74:563-572, 2004.
34) Takahashi M, Tanaka T, Takahashi H, Hoshino Y, Nagashima S, Jirintai, Mizuo H, Yazaki Y, Takagi T, Azuma M, Kusano E, Isoda N, Sugano K, Okamoto H. Hepatitis E Virus (HEV) strains in serum samples can replicate efficiently in cultured cells despite the coexistence of HEV antibodies: characterization of HEV virions in blood circulation. J Clin Microbiol 48:1112-11125, 2010.
35) Provost PJ, Wolanski BS, Miller WJ, Ittensohn OL, McAleer WJ, Hilleman MR. Physical, chemical and morphologic dimensions of human hepatitis A virus strain CR326 (38578). Proc Soc Exp Biol Med 148:532-539, 1975.
36) Lemon SM, Binn LN. Incomplete neutralization of hepatitis A virus in vitro due to lipid-associated virions. J Gen Virol 66:2501-2505, 1985.

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Cell culture system for hepatitis E virus

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Early studies reported propagation of hepatitis E virus (HEV) in primary hepatocytes or several established cell lines, but replication was inefficient. Recently, using inocula comprised of fecal suspensions with high loads of HEV, originally obtained from Japanese patients who contracted domestic infection of genotype 3 HEV (the JE03-1760F strain, 2.0 x 10^7 copies/ml) or genotype 4 HEV (the HE-JF5/15F strain, 1.3 x 10^7 copies/ml), we developed an efficient cell culture system for HEV in PLC/PRF/5 and A549 cells, which yielded the highest HEV load of 10^8 copies/ml in the culture supernatant, and we successfully propagated six or more generations in serial passages of culture supernatant. In addition, we constructed a full-length infectious cDNA clone (pJE03-1760F/wt) of the JE03-1760F strain, which can replicate efficiently in PLC/PRF/5 and A549 cells. Using a derivative ORF3-deficient (ΔORF3) mutant, we demonstrated that the ORF3 protein of HEV is responsible for virion egress from infected cells and is present on the surface of released HEV particles, which is associated with lipids. Various HEV strains in blood circulation were also propagated efficiently in PLC/PRF/5 and A549 cells. Our in vitro cell culture system can be used for propagation of a wide variety of HEV strains in feces and sera from various infected patients, allowing extended studies on viral replication specific to different HEV strains.

C E型肝炎
33 E型肝炎の輸入感染症例

自治医科大学感染・免疫学講座ウイルス学部門 岡本宏明

E型肝炎ウイルス(hepatitis E virus; HEV)は主として肝臓で増殖し，胆道を通って腸管に排出され，糞便とともに体外に排泄される．そのため，不十分な衛生環境下では，糞-口(fecal-oral)ルートでの感染が起こり易い．アジア，アフリカ，中米の熱帯・亜熱帯地域に位置する発展途上国では，河川の氾濫などによって飲み水が患者の糞便に由来するウイルスに汚染され，古くから大小様々な規模の流行性E型肝炎が繰り返し発生してきた．現在でもなお，このような水系感染に伴うE型肝炎は発展途上国での主たる風土病の一つであり，医学的にも公衆衛生学的にも問題となっている．このような侵淫地域に渡航し，不注意にも生あるいは加熱不十分な食べ物や飲料水を摂取することによってHEVに感染し，帰国後に発症するのが輸入感染症としてのE型肝炎である．侵淫国からの入国者がHEVに感染しながらも無症状のまま日本を訪れ，ウイルスを持ち込む場合もある．今世紀に入って，人獣共通感染症(zoonosis)としての国内感染型のE型肝炎がにわかに注目され，ややもすると海外渡航に起因する輸入HEV感染症例の存在が忘れられがちであるが，決してなくなったわけではない．アジア・アフリカへの渡航歴のある日本人，あるいはそれらの国々からの入国者が急性肝炎を発症した場合の鑑別診断の一つとして忘れてはならない疾患であることを本項で強調したい．

症　例

30歳，男性．

[主　訴] 全身倦怠感，食欲不振，右季肋部痛，黄疸．

[既往歴] 特記すべきことなし．

[現病歴] 本症例は2006年1月23日から中国雲南省，チベット自治区を経て3月10日にネパール・カトマンズに入った．4月30日にタイに移動してから濃厚尿，黄疸に気づき5月3日に帰国した．5月8日黄疸が増強し，急性肝炎として入院した．

[初診時所見] 身長165 cm，体重67.2 kg，体温36.2℃，血圧122 / 70 mmHg，脈拍61 / min，整．意識清明．

[家族歴] 特記すべき事項なし

[身体所見] 皮膚は黄染し，眼球結膜に黄疸を認めた．神経学的異常所見はない．表在リンパ節は触知されなかった．腹部触診では腫瘤は触れず，肝は右肋弓下に1横指触知し，辺縁は鋭，硬度は軟であった．