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.

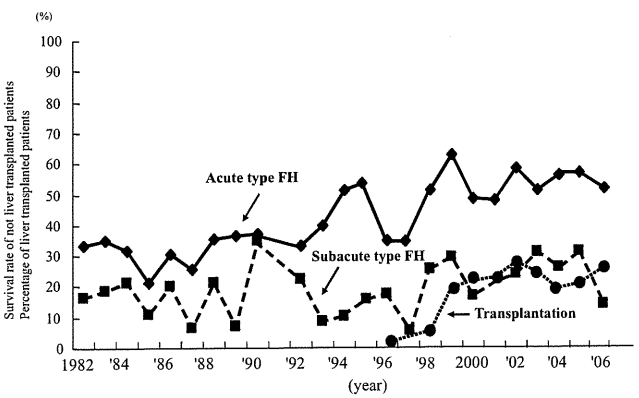

Figure 1 Survival rate of not liver transplanted patients with fulminant hepatitis(FH) and percentage of liver transplanted patients.

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

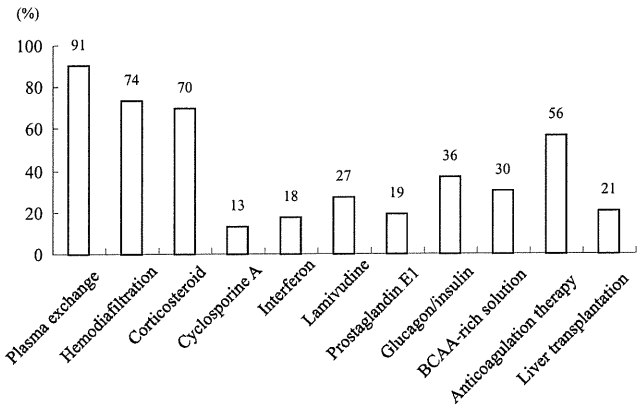

Figure 2 Percentage incidence of therapies performed for fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006). BCAA, branched-chain amino acid.

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.

Acknowledgments

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Technical note

Varicella-zoster virus-specific cell-mediated immunity in subjects with herpes zoster

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ABSTRACT

Though cell-mediated immunity (CMI) against varicella-zoster virus (VZV) is critical for prevention of the onset of herpes zoster (HZ), clinicians currently lack a simplified procedure to monitor CMI. We have recently developed an assay, called the IFN-γ release assay, and showed that it is a simple and reliable method to determine VZV-specific CMI. In the present study, we applied an IR assay to measure the VZV-specific CMI of patients with HZ. VZV-specific CMI levels were significantly high at the onset of the disease, but were decreased several weeks later. In contrast, CMI VZV-specific antibody titers increased in convalescent phase compared to those in acute phase. Thus, this technology is likely to be very useful in monitoring ongoing VZV-specific immune status.

1. Introduction

Varicella is the manifestation of a primary infection by the varicella-zoster virus (VZV). The virus maintains a lifelong latency in the neurons of the sensory ganglia, but can be reactivated as a consequence of declining VZV-specific cell-mediated immunity (CMI). It is generally accepted that herpes zoster (HZ or shingles) is caused by reactivation of the virus due to decreased CMI. Studies have shown that the frequency and severity of HZ increase in older patients and in those with hematopoietic stem cell transplants (Hope-Simpson, 1965; Gnann and Whitley, 2002; Hata et al., 2002), when VZV-specific CMI decreases (Levin et al., 2003). Moreover, the increased anti-VZV-CMI that occurs with HZ onset is associated with reduced HZ severity and reduced postherpetic neuralgia (Weinberg et al., 2009). These observations indicate the importance of VZV-specific CMI in the occurrence and prognosis of HZ.

Various methods have been developed to detect VZV-specific CMI. Among them, the lymphocyte proliferation assay, which measures lymphocyte propagation stimulated by the VZV antigen, has been most frequently used. Recently, an enzyme-linked immunospot (ELISPOT) assay has been used to estimate VZV-specific CMI (Levin et al., 2003). In this method, the number of cells producing the interferon-γ (IFN-γ) by antigen stimulation is calculated, and the results of this assay have been found to correlate well with those of skin tests (Sadaoka et al., 2008). Although the ELISPOT assay is relatively easy to perform compared with other in vitro methods, a simpler and more cost-effective method is necessary for detecting CMI during routine examinations in point-of-care settings.

To address this need, we previously validated another assay, the IFN-γ release assay (IR assay), which can be

Abbreviations: VZV, varicella-zoster virus; HZ, herpes zoster; CMI, cell-mediated immunity; ELISPOT, enzyme-linked immunospot; IR assay, interferon-γ release assay; PBMCs, peripheral blood mononuclear cells.

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used to easily determine VZV-specific CMI by using whole peripheral blood rather than peripheral blood mononuclear cells (PBMCs). Employing a commercially available VZV vaccine, we found that >100 pg/ml IFN-γ was produced from the whole peripheral blood of children who had immune memory to VZV, but not from that of children with no immune memory (Otani et al., 2009). Based on this result, in the present study, we used the same method to measure changes in VZV-specific CMI and antibody titers at both acute and convalescent phases in adult HZ patients. These measures were taken with the aim of elucidating the immune status of the disease.

2. Materials and methods

2.1. Study subjects

A total of 14 patients with HZ who visited the hospital of the Hyogo College of Medicine participated in the present study. The mean age of the donors was 66 years (range, 18–84 years); 6 (43%) of the participants were men. Most patients had underlying diseases such as cancer, autoimmune disease, or diabetes mellitus; however, 2 patients had no other diseases. All patients underwent clinical evaluation by a dermatologist, and blood samples were collected at the first consultation on rash onset (acute phase) in the patient and at the second consultation (convalescent phase) to evaluate alleviation of the disease.

This study was approved by the ethics committee of the Hyogo College of Medicine, and blood collection was performed after obtaining informed consent from all patients.

2.2. IR assay

The IR assay was performed as previously described (Otani et al., 2009). Briefly, freshly isolated, heparinized whole-blood cells were cultured with a VZV antigen in the form of ultraviolet-inactivated (2700J/m2) VZV vaccine (Oka strain, BIKEN, lot VZ050). Culture supernatants were collected 48 h after co-cultivation, and IFN-γ concentrations were quantified using an enzyme-linked immunosorbent assay (IFN-γ Assay Kit; Biosource International, Camarillo, CA) according to the manufacturer's instructions. Either phytohemagglutinin (2.5 μg/ml) or medium was added to the blood instead of the varicella vaccine as the positive or negative control, respectively.

2.3. Antibody titration

Anti-VZV antibody titers in the sera were examined by an indirect, immunofluorescence test (Otani et al., 2009). Serially diluted sera were spotted onto VZV (Oka vaccine strain)-infected MRC-5 cells, which had been fixed with acetone on the slides, and incubated at 37 °C for 1 h. After the slides were washed, fluorescein-conjugated goat antibodies against human IgG were added. The slides were again washed following incubation at 37 °C for 1 h, and signals were detected using a fluorescence microscope. The antibody titers were determined as the highest dilution of serum showing a positive signal.

3. Results

3.1. IR assay of blood from HZ subjects

As shown in Fig. 1a, marked amounts (>100 pg/ml) of IFN-γ were released from the whole blood of all patients, except 1, in the acute phase of HZ. However, except in the case of 1 patient, the production from whole blood in the convalescent phase (1–8 weeks after the acute phase) substantially decreased. The amount of IFN-γ released at HZ onset did not correlate with HZ severity.

3.2. VZV-specific antibody titer

At the first consultation, all patients had detectable anti-VZV IgG antibodies, and the titers had increased more than 4-fold in 11 of 14 patients at the second consultation (Fig. 1b), indicating a slower response of the antibody than to of CMI (Fig. 1a).

4. Discussion

The present study was undertaken to evaluate VZV-specific CMI responses of HZ patients by measuring IFN-γ production from whole blood stimulated with the VZV antigen. The results suggest that VZV-specific CMI may increase prior to and peak around the time of rash onset through virus reactivation and then decline. Similar kinetic patterns

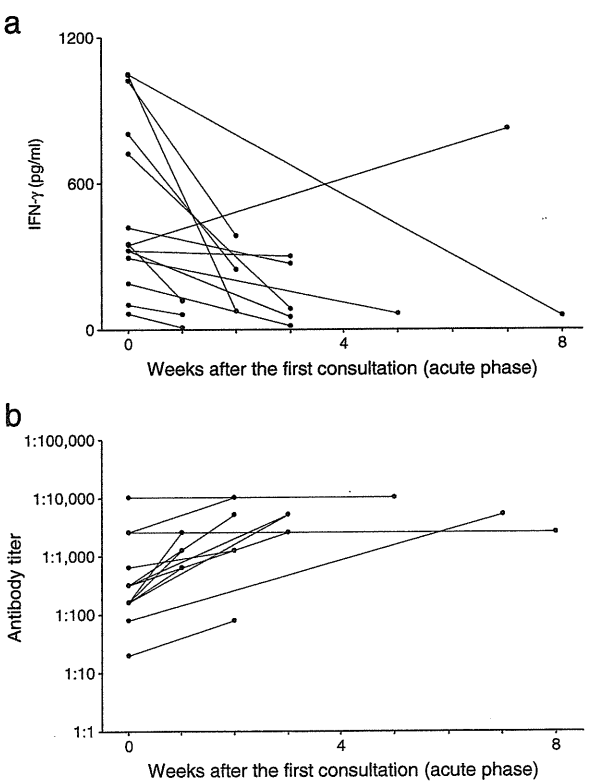

Fig. 1. (a) IFN-γ production and (b) antibody titers of 14 subjects at the acute and convalescent phases. Because 2 subjects exhibited identical antibody responses (from 1/320 to 1/5120), 13 reaction patterns are seen in (b).

for anti-VZV CMI in HZ patients have also been demonstrated using the IFN-γ ELISPOT assay (Weinberg et al., 2009), with results showing that VZV-specific effector T-cell responses rose quickly and peaked 1–3 weeks after the onset of HZ rash, followed by a rapid decay 3–6 weeks later. Thus, the IR assay results are consistent with those of the IFN-γ ELISPOT assay, and IR assay could therefore be substituted for that method.

Regarding the kinetics of anti-VZV antibodies, similar to the present results, titers recorded several weeks after the onset of HZ rash have been shown to be much higher than the titers recorded within 1 week of rash onset (Weinberg et al., 2009). Because the antibody responses lag behind disease progression, measurement of CMI is crucial for identifying ongoing immune status and for predicting prognosis.

After natural infection or vaccination with live vaccine both VZV-specific antibody and CMI are induced (Weinberg et al., 2009), resulting in the restriction of the viruses in sensory neurons. It is thought that the essential component responsible for protection against HZ is VZV-specific CMI, which declines progressively with advancing age. This is because the common, age-related decrease in VZV-specific T cells is associated with an increased risk of HZ (Hope-Simpson, 1965; Gnann and Whitley, 2002; Levin et al., 2003), but levels of VZV-specific antibody do not decline with age (Weinberg and Levin, 2010). In contrast, congenital and acquired agammaglobulinemias are not associated with increased risk of HZ, and administration of an antibody to VZV does not ameliorate the marked increase in the frequency of HZ (Weinberg and Levin, 2010). Despite these findings suggesting low levels of VZV-specific CMI, however, the precise role of the CMI remains unclear in most cases. The lack of a simple and reliable method to measure VZV-specific CMI is the apparent cause of this unclear picture.

In this study, blood from 1 patient produced a modest amount of IFN-γ (<100 pg/ml) in the acute phase, and it further declined in the convalescent phase. We previously demonstrated that >100 pg/ml IFN-γ was produced in the blood of children who had immune memory to VZV, but that non-immune children produced lower amounts of IFN-γ. Yawn et al. (2011) have recently shown that the population-based recurrence rate of HZ was 6.2% after 8 years of follow-up, a higher incidence than that previously reported. Long-term follow-up of virus-specific CMI in a large sample of HZ patients is essential to determine the threshold level of IFN-γ production necessary for protection from a second case of HZ. It will be especially noteworthy to find if patients with low IFN-γ production experience a second case of HZ shortly after the first episode. Our method will contribute to clarifying these issues.

Using the IFN-γ ELISPOT assay, one can quickly obtain very sensitive, reproducible, and reliable results for VZV-specific CMI. However, this method has several disadvantages. First, it requires significant sample processing, including PBMC preparation and cell counting. Second, even though a small volume of blood (3–5 ml) per person is required, this volume has been identified as a critical factor in very young children and infants. Third, the PBMCs must be utilized for the experiment within a few hours after collecting blood from the subjects, creating time-pressured situations. Although cryopreserved PBMCs could also be used, it has been shown that these produce lower amounts of IFN-γ than do fresh PBMCs (Weinberg et al., 2009). In contrast, the IR assay, which requires as little as 1 ml of blood per person, does not require PBMC preparation. Omission of this step, coupled with the use of commercially available VZV vaccine as an antigen for PBMC stimulation, dramatically simplifies the use of the assay, suggesting that it is more appropriate for use in point-of-patient-care settings than the ELISPOT assay.

In conclusion, in the present study, we applied the IR assay to measure VZV-specific CMI levels in HZ patients and obtained results demonstrating that this technology is likely to be very useful in monitoring ongoing VZV-specific immune status.

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