Short reports

Human herpesvirus 6 latently infects mononuclear cells but not liver tissue

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Abstract

Aim—To investigate whether human herpesvirus 6 (HHV-6) can cause latent infection of liver tissue.

Methods—Peripheral blood and liver tissue were collected from 25 living related liver transplant recipients at the time of transplantation. An avidin-biotin complex peroxidase method was used to identify HHV-6 antigen in the liver tissue. A nested polymerase chain reaction (PCR) was used to detect HHV-6 DNA in the liver tissue and mononuclear cells. Variant of HHV-6 was determined by the presence of the Hind III site in a second PCR product.

Results—Immunohistochemical analysis for HHV-6 antigen was negative in all the liver specimens. HHV-6 DNA was not detected in liver tissue. Virus DNA was detected in peripheral blood mononuclear cells in nine of 25 recipients. All nine HHV-6 identified in the mononuclear cells were variant B.

Conclusions—HHV-6 variant B latently infects mononuclear cells but not liver tissue.

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Keywords: mononuclear cells; liver transplantation; polymerase chain reaction; human herpesvirus 6

Human herpesvirus 6 (HHV-6) is a recently discovered member of the human herpesvirus family.¹ On the basis of genomic DNA sequences, cell tropism, and reactivity to several monoclonal antibodies, two distinct variants (variant A and variant B) have been described. Although primary infection with variant B HHV-6 causes exanthem subitum,² the clinical features of variant A HHV-6 infection remain unclear. The virus probably latently infects the body after the primary infection and then reactivates in an immunosuppressed state like other human herpesviruses. HHV-6 has recently been recognised as an opportunistic pathogen in transplant recipients.³ Since infection with the virus after organ transplantation could be fatal,⁴ it is important to prevent it. The route of transmission of the virus is one of the important issues to resolve in controlling infection after transplantation. A recent paper suggests that

Table 1 Characteristics of the patients

Age (months)	92 (range 4 to 390)
Sex (M/F)	11/14
Underlying disease	
Biliary atresia	21
Wilson disease	2
Tyrosinaemia	1
Fulminant hepatic failure	1

HHV-6 is transmitted by the donor liver graft during transplantation.⁵ Therefore, it is very important to determine whether HHV-6 can latently infect liver tissue.

In this report, 25 patients with end stage liver disease were studied to determine whether the HHV-6 genome was present in their liver tissue and peripheral blood mononuclear cells and whether HHV-6 antigen could be detected in their liver tissues. We showed that HHV-6 persists in peripheral blood mononuclear cells but not in liver tissue.

Methods

We studied 25 patients who received liver transplants from living relatives in Kyoto University Hospital because of end stage liver disease. The characteristics of the patients with regard to age, sex, and underlying disease are summarised in table 1. The median age of the recipients was 92 months (range 4-390) at the time of transplantation. No immunosuppressive drugs were given before transplantation in any of the recipients.

Peripheral blood and liver tissues were collected from the recipient at the time of transplantation. Each liver specimen was routinely fixed in 4% formaldehyde in phosphate buffered saline and embedded in paraffin. Serial sections were analysed by immunohistochemical and polymerase chain reaction (PCR) techniques. Peripheral blood mononuclear cells and plasma were separated by Ficoll-Hypaque gradient. Cells were used for detection of HHV-6 DNA by the PCR assay, and plasma was used for measurement of HHV-6 antibody titres.

To detect the virion protein of variant B HHV-6, we used a variant specific monoclonal antibody (C3108-103) which recognises a 101 kDa protein of variant B HHV-6. The antibody, which does not cross react with the homologous protein of variant A HHV-6,⁶ was

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Table 2 Results of human herpesvirus 6 (HHV-6) antigen and DNA determinations in each patient

Case	Age (months)	Sex	HHV-6 antibody titre	Antigen in liver tissue	DNA in MNC	DNA in liver tissue
1	62	F	64	N	N	N
2	186	F	64	N	N	N
3	9	F	8	N	N	N
4	10	F	<8	N	N	N
5	125	M	64	N	P	N
6	6	F	128	N	P	N
7	121	M	32	N	N	N
8	14	F	32	N	N	N
9	175	M	64	N	P	N
10	8	F	8	N	P	N
11	320	F	>256	N	P	N
12	10	M	8	N	N	N
13	13	M	>256	N	N	N
14	4	F	<8	N	N	N
15	21	F	32	N	P	N
16	9	F	8	N	N	N
17	234	M	32	N	N	N
18	12	M	8	N	N	N
19	390	F	128	N	P	N
20	278	F	64	N	N	N
21	19	M	64	N	P	N
22	168	M	32	N	P	N
23	56	F	128	N	N	N
24	48	M	32	N	N	N
25	7	M	8	N	N	N
Positive rate (%)				0	36	0

MNC, mononuclear cells; N, negative; P, positive.

kindly provided by Dr Philip Pellett (Center for Disease Control and Prevention, Atlanta, Georgia, USA). Immunohistochemical analysis was performed on paraffin sections by an avidin-biotin complex (ABC) peroxidase method.

Sections of liver tissue were cut from the paraffin blocks and deparaffinised by xylene. Paired samples of mononuclear cells and liver tissue were lysed overnight in lysis buffer (10 mM Tris HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 0.4% sodium dodecyl sulphate, 1 mg/ml proteinase K) at 65°C. DNA was extracted by phenol-chloroform treatment followed by ethanol precipitation. A 1 µg aliquot of the total DNA (quantified spectrophotometrically) extracted from each sample was used for PCR. Nested PCR was carried out for amplification of HHV-6 DNA by using two primer sets (A/C, HS6AE/HS6AF) as previously described.⁷ The primers finally amplified a DNA fragment of 751 base pairs of a putative large tegument protein gene. The sensitivity of the PCR assay was determined by using serial dilutions of plasmid pSTY-05 (kindly provided by Dr K Yamanshi, Department of Bacteriology, Osaka University, Osaka, Japan). We were able to detect 100 copies of the virus genome. The type of HHV-6 was determined by the presence of a Hind III site in each second PCR product. A Hind III site was shown to be present in the type B but not the type A variant of HHV-6. Aliquots of TE buffer (Tris HCL 10 mM plus EDTA 1 mM) were processed as a negative control, and appropriate precautions to avoid cross contamination were taken during sample preparation and while performing the PCR. All liver tissue specimens were analysed using a primer to the β globin gene to confirm the absence of residual PCR inhibitors in the sample.

IgG antibody titres to HHV-6 were measured by an indirect immunofluorescence assay as described elsewhere.⁸

Results

Details of each subject and the results of the experiments are summarised in table 2. All the recipients except for cases 4 and 14 had an antibody to HHV-6 at the time of transplantation at titres ranging between < 8 and > 256. In liver tissue, immunohistochemical analysis for HHV-6 antigen proved negative in all specimens. Moreover, none of the liver tissues contained HHV-6 DNA on the basis of our PCR assay. However, the β globin gene was amplified in all the liver tissue specimens. To check the reliability of our PCR assay, HHV-6 DNA was amplified from a sample of HHV-6 infected cord blood mononuclear cells, which were fixed with formaldehyde and embedded in paraffin.

HHV-6 DNA was detected in peripheral blood mononuclear cells obtained from the cases 5, 6, 9, 10, 11, 15, 19, 21, and 22. HHV-6 DNA was thus detected in peripheral blood mononuclear cells in 36% of the recipients (9/25) at the time of transplantation. As shown in fig 1, all nine HHV-6 detected in peripheral blood mononuclear cells were variant B.

Discussion

The first case of HHV-6 infection after liver transplantation, which was reported by Ward *et al*,⁵ showed seroconversion after the operation. These investigators suggested that the virus was transmitted by the donor liver. We have recently experienced a case of a six week old boy from whom HHV-6 was isolated after liver transplantation. According to sero-epidemiological data for Japan,⁸ it is very unusual to acquire a primary infection with the virus by six weeks after birth. In this recipient, the donor liver appeared to be the source of HHV-6 infection.

Figure 1 Agarose gel of Hind III digested second PCR products for evaluation of the variant of the virus. Lanes 1–9: positive second PCR products; all samples were digested with the enzyme. Lane 10: control for variant B HHV-6 (FG-1 strain). Lane 11: control for variant A HHV-6 (DA strain).

Ideally, in order to determine whether donor liver tissue could be the source of transmission of HHV-6 between donor and recipient, we should have examined the donor liver tissue and peripheral blood mononuclear cells. However, it was impossible to obtain samples of donor graft at the time of transplantation, because all the liver transplants were from healthy relatives. In contrast to the donor graft, it was easy to obtain recipient liver tissue specimens after the total resection of the recipient liver. Therefore our study design involved examining liver tissue and mononuclear cells obtained simultaneously from the recipient. This enabled us to determine whether HHV-6 can latently infect liver tissue, assuming that the characteristics of HHV-6 infection in child and adult liver tissue are comparable.

There are data suggesting that HHV-6 persists latently in salivary glands, lymph nodes, kidney cells, and monocytes/macrophages. The Kupffer cell in the liver is closely related to the monocyte/macrophage. Thus it is conceivable that HHV-6 could persist in donor liver tissues. However, we did not detect HHV-6 antigen or DNA in any of our recipients' liver tissues, despite taking careful precautions to eliminate the possibility of inhibitors to our PCR assay. These results suggest that HHV-6 does not latently infect liver tissue. However, we cannot exclude the possibility that small amounts of the virus, below the detection limit of our PCR assay, could persist in liver tissue. This issue can only be resolved by identifying virus DNA using in situ PCR.

Variant B HHV-6 DNA was detected in peripheral blood mononuclear cells of nine of the 25 recipients (36%). This is a lower rate than in previous studies, all of which have been in healthy adults, while our analysis involved children with end stage liver disease. The discrepancy between the results of our study and the previous investigations might depend on the age difference in the subjects and the sensitivity of the PCR assay. From the results of this experiment and those of immunohistochemical and PCR analyses of liver tissue, it is likely that HHV-6 can persist in peripheral

blood mononuclear cells but not in liver tissue. However, liver tissue may contain small numbers of mononuclear cells that are latently infected with HHV-6.

The HHV-6 variant was determined by restriction enzyme digestion of PCR amplicates in all nine infected recipients. They all carried the HHV-6 B variant. We have recently demonstrated variant B HHV-6 viraemia after liver transplantation. According to other reports, HHV-6 variant B seems to be more prevalent in liver transplant recipients than variant A.^{9,10} The reason for this difference is unknown at present. We need to clarify the epidemiology and clinical features of variant A infection.

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- 1 Salahuddin SZ, Ablashi DV, Markham PD, *et al.* Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* 1986;234:596-601.
- 2 Yamanishi K, Okuno T, Shiraki K, *et al.* Identification of human herpesvirus-6 as a causal agent for exanthem subitum. *Lancet* 1988;i:1065-7.
- 3 Yoshikawa T, Suga S, Asano Y, *et al.* Human herpesvirus-6 infection in bone marrow transplantation. *Blood* 1991;78:1381-4.
- 4 Drobyski WR, Knox KK, Majewski D, *et al.* Brief report: fatal encephalitis due to variant B human herpesvirus 6 infection in a bone marrow-transplant recipient. *N Engl J Med* 1994;330:1356-60.
- 5 Ward KN, Gray JJ, Efsthathiou S. Brief report: primary human herpesvirus 6 infection in a patient following liver transplantation from a seropositive donor. *J Med Virol* 1989;28:69-72.
- 6 Pellett PE, Sanchez-Marintez D, Dominguez G, *et al.* A strongly immunoreactive virion protein of human herpesvirus 6 variant B strain Z29: identification and characterization of the gene and mapping of variant specific monoclonal antibody reactive epitope. *Virology* 1993;195:521-31.
- 7 Suga S, Yazaki T, Kajita Y, *et al.* Detection of human herpesvirus 6 DNAs in samples from several body sites of patients with exanthem subitum and their mothers by polymerase chain reaction assay. *J Med Virol* 1995;46:52-5.
- 8 Yoshikawa T, Suga S, Asano Y, *et al.* Distribution of antibodies to causative agent of exanthem subitum (human herpesvirus-6) in healthy individuals. *Pediatrics* 1989;84:675-7.
- 9 Singh N, Carrigan D, Gayowski T, *et al.* Variant B human herpesvirus-6 associated febrile dermatosis with thrombocytopenia and encephalopathy in liver transplant recipient. *Transplantation* 1995;60:1355-7.
- 10 Schmidt CA, Wilborn F, Weiss K, *et al.* A prospective study of human herpesvirus type 6 detected by polymerase chain reaction after liver transplantation. *Transplantation* 1996;61:662-4.