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A Serological Investigation of Human Herpesvirus 6 Infections in Liver Transplant Recipients and the Detection of Cross-Reacting Antibodies to Cytomegalovirus

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Sera from 50 orthotopic liver transplant recipients were examined for antibodies to human herpesvirus 6 (HHV-6) and cytomegalovirus (CMV), and the findings correlated with the clinical condition of the patients. Both primary and secondary HHV-6 infections were detected serologically following liver transplantation. Interpretation of serological assays is complicated by CMV and HHV-6 antibody cross reactions which were common. Sera from 5 patients became HHV-6 antibody negative following absorption with CMV infected cells. Thirty patients were initially seronegative for HHV-6 antibodies, 12 remained so following transplantation, 5 developed cross reacting antibodies, and 13 seroconverted. The seroconversions occurred at 4 to 8 weeks post-transplant in the same time period as CMV antibody rises. HHV-6 IgM was detected in only 4 of the 13. Of the 7 patients who had serological evidence of active HHV-6 infections but no evidence of CMV infection, 4 (56%) had fever, 1 (14%) hepatitis, 1 (14%) lung dysfunction, and 3 (42%) neurological disorders. In the 12 patients who remained HHV-6 antibody negative, there were fewer fevers and neurological disorders.

KEY WORDS: human herpesvirus 6, cytomegalovirus, liver transplantation

INTRODUCTION

Human herpesvirus 6 (HHV-6) was first isolated from lymphocytes of patients with lymphoproliferative disorders [Salahuddin et al., 1986] and HIV infection [Tedder et al., 1987]. It has been serologically associated with roseola infantum [Yamanishi et al., 1988], and HH6 antibody has been detected at high frequency in the general population [Briggs et al., 1988]. HHV-6 has been said to cause hepatitis in a 25-year-old woman [Dubedat and Kappagoda, 1989]. It is to be expected that, as with other herpesviruses, HHV-6 may cause both primary and reactivated infections in post-transplant recipients and this has been demonstrated in renal transplant recipients [Morris et al., 1989]. Serological investigations of HHV-6 infections may be complicated by possible cross reactions with cytomegalovirus (CMV) to which it is genomically related [Efstathiou et al., 1988]. We report a retrospective serological study of orthotopic liver transplant recipients in which we have identified primary and secondary HHV-6 infections post-transplant, and correlated the antibody rises with the clinical findings in the patients. We investigated specifically possible CMV/HHV-6 antibody cross reactions.

MATERIALS AND METHODS

Sera

A total of 220 sera from 50 liver transplant recipients were tested retrospectively for HHV-6 antibody. All patients had 2 or more sequential sera tested (range 2 to 13). All sera were preabsorbed overnight (O/N) at 4°C or for 1 hour at room temperature (RT) with acetone fixed uninfected J JHAN cells before being tested blind for HHV-6 IgG antibody at a dilution of 1:50 in phosphate buffered saline. Selected sera were tested for HHV-6 IgM at a dilution of 1:12 after preabsorption with Gullsorb reagent (Gull Laboratories, USA) for 15 minutes at RT to remove IgG. To examine possible cross reactions with CMV, selected sera were retested for HHV-6 IgG following absorption with acetone fixed CMV infected fibroblasts and/or HHV-6 infected J JHAN cells. Absorptions were at 4°C O/N.

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Virus

HHV-6 in J JHAN cells was kindly supplied by R.S. Tedder of University College and Middlesex Medical School, London.

Cells

The T-lymphocyte cell line J JHAN was infected with HHV-6 virus and cultured for from 2 to 3 weeks until a good CPE developed. Infected cells were washed in PBS, placed on multispot slides, dried and fixed in acetone, then stored at −20°C until required. Uninfected J JHAN cells were prepared in the same way.

Assays

HHV-6 IgG. After absorption, diluted sera were incubated with test and control cells on multispot slides for 30 minutes at RT. After washing, FITC conjugated rabbit antihuman IgG (Dako) was added and incubated at RT for 20 minutes.

HHV-6 IgM. Similar to the above, but the serum incubation continued for 3 hours and the anti-IgM continued for 1 hour. Appropriate controls were included with each test run, and wells were read on an incident-light-fluorescence microscope. They were scored −, ±, +, ++, or +++.

Clinical Data

Details of the patients symptoms and therapy, and results of other laboratory tests such as liver function tests (LFTs) and liver biopsy findings, were obtained from the hospital notes of the 31 patients on whom we had early sera. One of these had 2 transplants included in the study.

RESULTS

Serological

HHV-6 antibody was detected in the sera of 35 of the 50 (70%) liver transplant recipients in the period of 5 to 12 weeks post-transplant. The 31 patients who had early and late sera could be categorised in 4 groups according to their early CMV/HHV-6 antibody status. Group 1 comprised 8 patients initially seronegative for both viruses (Table I), and Group 2 contained 16 who had CMV antibody but no HHV-6 antibody (Table II). In Group 3 were 2 with HHV-6 but no CMV antibody, and in Group 4, 5 patients were seropositive for both viruses.

Overall, of 25 cases seronegative for HHV-6 at or near transplant, 12 (48%) remained seronegative and 13 (52%) seroconverted. The reactivation rate, as measured by rising antibody in the 7 HHV-6 antibody positive patients, was 100%. The equivalent rates for CMV were 6/10 (60%) seroconversions and 16/21 (76%) secondary infections. HHV-6 IgM was investigated in 12 patients with presumed primary HHV-6 infection and detected in only 3. An additional patient had HHV-6 IgM detected elsewhere [Ward et al., 1989].

Cross-Reacting Antibodies

Five patients developed HHV-6 cross-reacting antibodies which could be removed by absorption with CMV. These occurred in both primary (2 of 6) and secondary (3 of 15) CMV infections. False positive CMV infections were not investigated.

The HHV-6 antibody rises occurred at 3 to 8 weeks post-transplant, but most were detected at 4 to 5 weeks. In 2 patients, R and C (Fig. 1), rises were delayed beyond 7 weeks. R had cross-reacting antibody and C had an HHV-6 seroconversion. Both had received gancyclovir therapy starting at 3/52 (R) and 4/52 (C) post-transplant.

Clinical

Of the 31 patients for whom clinical data were included, unexplained fever occurred in 31%, hepatitis in 31%, chest infections in 16%, and neurological disorders in 22% (Table III). Eight of the 31 patients died, all except one, at or beyond 3/12 after transplant; 4 died early after second transplants. In none of these was primary HHV-6 infection implicated temporally, but a rising titre of antibody to HHV-6 occurred in one patient (LW) who died at 5 weeks post transplant with fever, brain, and lung disorders. The brain disorder started at 3½ weeks post-transplant, was diagnosed as central pontine myelinolysis and was associated with fever, pleural effusion, and lung consolidation.

Only 1 patient with presumed HHV-6 primary infection remained seronegative for CMV. About 7 weeks post-transplant, he developed fever and numbness of the lateral aspect of the left thigh. This was mild and resolved within a few days. By week 8 post-transplant he became HHV-6 antibody positive.

There were 6 other patients with serological evidence of active HHV-6 infections, but no significant change in CMV antibody. Of these 7 patients, 4 (56%) had fever, 1 (14%) had lung infection, and 3 (42%) had neurological disorders (Table IV). When compared with the 12 HHV-6 negative patients (Table III), there appears to have been an increase in unexplained fevers and neurological disorders but no increase in hepatitis.

Hepatitis occurred in 10 of 31 patients. In 5 patients it was due to CMV, in 1 to Hepatitis B virus, and in 1 was thought to be an early recurrence of the NANB infection that had preceded the transplant. The 3 other patients all had active CMV and HHV-6 infections, and the hepatitis could have been caused by either or both viruses. Six of the patients with HHV-6 antibody rises were treated with gancyclovir. In one of these, treatment occurred before the onset of the rise and may have been responsible for the delayed rise in this patient. Another patient with a "false" antibody rise showed a similar delay.

DISCUSSION

We have shown that both primary and secondary HHV-6 infections can be detected serologically following liver transplantation. Since the study was partly designed to investigate HHV-6/CMV cross-reactions,

TABLE I. Serological Responses at 6 to 9 Weeks Post-Liver Transplantation in 8 Patients Seronegative for CMV (<1:4) and HHV-6 (<1:50) in the Early Weeks Post Transplant

	CMV antibody			HHV-6 antibody					
Patients	Early CF titre	At 6-9 weeks CF titre	IgM	Early IgG	At 6-9 weeks IgG	IgM	Post CMV absorption	Post HHV-6 absorption	Comment
PA	<4	<4		−	−				CMV− HHV-6−
SM	<4	<4		−	−				CMV− HHV-6−
SB Tx (1)	<4	<4		−	−				CMV− HHV-6−
Tx (2)	<4	8	++	−	++	−	−		CMV+ HHV-6−
SR	<4	128	++	−	+		−		CMV+ HHV-6−
JF	<4	<4		−	+	−	+	−	CMV− HHV-6+
MN	<4	512	++	−	−				CMV+ HHV-6−
BC	<4	8	++	−	+	−	+	−	CMV+ HHV-6+
AA	<4	<8	+	−	+++	+	+	+	CMV+ HHV-6+

TABLE II. Serological Responses at 6 to 12 Weeks Post Transplant in 16 Patients Seropositive for CMV and Seronegative for HHV-6 in the Early Weeks Post Transplant

	CMV antibody			HHV-6 antibody					
Patients	Early CF titre	At 6-12 weeks CF titre	IgM	Early IgG	At 6-12 weeks IgG	IgM	Post CMV absorption	Post HHV-6 absorption	Comment
MG	8	16	−	−	−				CMV− HHV-6−
BT	16	>256	−	−	−				CMV+ HHV-6−
CR	8	32	+	−	−				CMV+ HHV-6−
MW	16	16	−	−	+	−	+		CMV− HHV-6+
VH	32	32	−	−	++	−	+	+	CMV− HHV-6+
MM	8	16	−	−	++	−	±		CMV− HHV-6+
SR	32	512	+	−	+	(+)			CMV+ HHV-6+
SA	8	128	−	−	++	±	+	−	CMV+ HHV-6+
AF	<8	512	+	−	++	±	+	+	CMV+ HHV-6+
XP	32	512	−	−	+	−	+	±	CMV+ HHV-6+
RA	8	>512	−	−	++	−	+		CMV+ HHV-6+
RTb	16	128	−	−	++	−	+		CMV+ HHV-6+
RTa	<8	32	−	−	+	−	±		CMV+ HHV-6+
SB	32	256	+	−	+		−		CMV+ HHV-6−
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NH	16	64	−	−	+	−	−		CMV+ HHV-6−

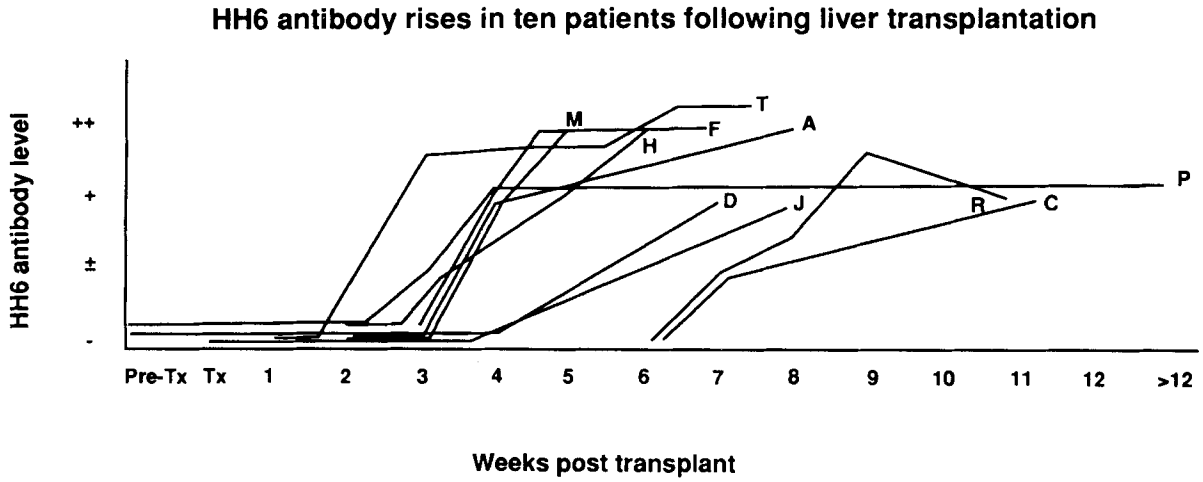

Fig. 1. HH-6 antibody levels detected by immunofluorescence in serial serum samples from 10 patients following liver transplantation.

TABLE III. Correlation of Clinical Findings With HHV-6 Antibody Status in the First 3 Months Following 32 Liver Transplants in 31 Patients*

Serological outcome	Total	Fever	Hepatitis	Lung problem	Neurological[a] disorder	Other
HHV-6 negative	12	2 (16%)	4 (33%)	2 (16%)	2 (16%)	3 (24%)
HHV-6 seroconversion	13	5 (39%)	4 (31%)	0	3 (24%)	3 (24%)
HHV-6 antibody rise	7	3 (42%)	2 (28%)	3 (42%)	2 (28%)	2 (28%)
	32	10 (31%)	10 (31%)	5 (16%)	7 (22%)	8 (25%)

*Excludes episodes in which bacterial infection was proven, response to antibiotics was obtained, and/or non-infectious cause was known.
[a]Most of these were diagnosed clinically as cyclosporin A toxicity.

TABLE IV. Clinical Findings in 31 Patients (32 Transplants) Following Liver Transplantation

Early serological status	Total	Outcome	No.	Clinical abnormalities[a] Fever	Hepatitis	Lung	Neurological	Other
HHV-6− CMV−	9	HHV-6− CMV−	3	0	1	1	0	0
		HHV-6− CMV+	3	1	2	1	0	2
		HHV-6+ CMV−	1	1	0	0	1	0
		HHV-6+ CMV+	2	1	2	0	0	0
HHV-6− CMV+	16	HHV-6− CMVP[b]	1	1	0	0	1	0
		HHV-6− CMV+	5	0	1	0	1	1
		HHV-6+ CMVP	3	1	0	0	1	1
		HHV-6+ CMV+	7	2	2	0	1	2
HHV-6+ CMV−	2	HHV-6+ CMV−	1	0	1	0	0	0
		HHV-6+ CMV+	1	1	1	1	0	0
HHV-6+ CMV+	5	HHV-6+ CMVP	2	2	0	1	1	0
		HHV-6+ CMV+	3	0	1	1	1	2
		Total	32	10	11	5	7	8

[a]Excludes episodes in which bacterial infection was proven, response to antibiotics was obtained, and/or non-infectious cause was known.
[b]P = Past.

there is a selection bias towards patients who were HHV-6 seronegative pre-transplant. Thus these results do not reflect the true prevalence of HHV-6 infection in liver-transplant recipients.

Serological investigation of HHV-6 in the post-transplant period is complicated by antibody cross reactions with CMV. In 5 patients these HHV-6 antibodies could be completely removed by absorption with CMV infected cells and thus were regarded as "false HHV-6 antibodies." Seroconversion was regarded as genuine in 13 of 24 HHV-6 negative patients. This gives a rate of 54%, which is similar to the rate of 60% for CMV seroconversion in the 10 recipients CMV negative at transplant. However, it was surprising that HHV-6 IgM was detected in so few of the HHV-6 seroconversions. This may indicate that our IgM assay was insensitive or that these are not primary infections. A decline in HHV-6 antibody prevalence with age has been noted by Brown and colleagues and would be consistent with the interpretation that not all seroconversions are primary infections.

Interpretation of clinical data is very difficult due to the multifactorial complexities in the post transplant period. Unexplained fever was more common in those who seroconverted to HHV-6 than in the HHV-6 seronegative group (39% vs. 16%, Table III), but hepatitis was similar in frequency in both groups. However some of these unexplained fevers could have been due to CMV. In the 7 patients with evidence of active HHV-6 but absent or quiescent CMV, fever and neurological disturbances did seem to occur more frequently (Table III): fever occurred in 4 (56%) and neurological problems in 3 (43%), compared with fever (16%) and neurological symptoms (16%) in 12 patients without evidence of active HHV-6 infection.

Hepatitis was not seen in any of the patients with HHV-6 infections only. In 3 patients with both CMV and HHV-6 infections, however, the hepatitis may have been caused by HHV-6 since, in two of these, the hepatitis occurred while the patient was seroconverting and, in the third, while there was a marked rise in the HHV-6 specific antibody titre. However, CMV may also have played a role in causing the hepatitis.

A total of 7 patients had neurological disturbances. Two of these episodes occurred in patients who remained negative for HHV-6 (2/12, 17%), whereas 5 occurred in HHV-6-positive patients (5/20, 25%). Three of the 5 were diagnosed as cyclosporin A toxicity. One of those has already been reported [Ward et al., 1989] in the literature, and the possible link of the patient's seizures with primary HHV-6 infection had been made. The 2 non-cyclosporin A related episodes were described above. Although there was a temporal link between the neurological disturbance in each of these

patients and rising antibody to HHV-6, further work on a larger sample will be necessary before such a link could be definitely made.

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