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ORIGINAL ARTICLE

# Demonstration of HHV-6 antigens in biopsies of kidney transplant recipients with cytomegalovirus infection

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## Summary

The activation of human herpesvirus-6 (HHV-6) commonly coexists with that of cytomegalovirus (CMV) in organ transplant recipients. No data exist of HHV-6 in renal allografts, whereas persistent CMV in the kidney associates with poor outcome and histopathologic changes. We examined HHV-6 and CMV antigens from kidney transplants with previous CMV infection. HHV-6 and CMV pp65 antigens were demonstrated by monoclonal antibodies and immunohistochemistry from 22 kidney transplants with previous CMV infection. CMV was diagnosed by antigenemia tests and/or viral cultures. HHV-6 antigens were found in 7/22 biopsies 18–1330 days after CMV infection, in infiltrating leukocytes in six, and in tubular epithelial cells in two patients. CMV infections were treated, and no virus could be detected from urine or blood thereafter, or at the time of the biopsy. Only 1/7 of these biopsies demonstrated also CMV antigens, whereas CMV antigens were found in 6/15 of the biopsies with no HHV-6. HHV-6 in the graft was associated with previous acute rejections, but not with any histopathological changes or reduced renal function. In conclusion, HHV-6 was a common finding in late renal allograft biopsies of patients with previous CMV infection, but its significance remains to be elucidated.

## Introduction

In organ transplant recipients receiving immunosuppressive medication, highly seroprevalent human herpesvirus-6 (HHV-6) reactivates from latency during the first post-transplant weeks, often together with cytomegalovirus (CMV) infection [1,2]. Whereas CMV has been associated with significant morbidity in organ transplant recipients and has also been associated with enhanced rejection of parenchymal organ transplants [3–6], the role of HHV-6 in pathological processes after transplantation is less clear. Most HHV-6 reactivations are caused by the HHV-6B and are asymptomatic, but HHV-6 infection has also been associated with fever and several neurologic symptoms after transplantation [7], and some studies have shown an association of HHV-6 with graft dysfunction and acute rejections after liver transplantation [8,9]. However, the role of HHV-6 in kidney transplant rejection is unknown.

Cytomegalovirus is able to persist in the kidney allograft for a long period after symptomatic infection and viremia, and this persistence has been associated with chronic inflammatory response and histopathologic changes in the graft and poor outcome [10,11]. Although some studies have reported the presence of HHV-6 in the renal allograft [12–14], not much is known about the HHV-6 in the kidney. We examined the presence and the possible role of HHV-6 and CMV in the biopsies of adult kidney transplant recipients with previous CMV infection.

## Patients and methods

Frozen biopsy material for the retrospective detection of HHV-6 and CMV antigens was available from 22 adult

kidney transplant recipients, transplanted during 1992–2000 in the Helsinki University Hospital, who all had had a previous CMV infection. Altogether 59 patients had both a previous CMV infection and a 6-month protocol biopsy taken during 1992–2000. All the 22 patients with available frozen biopsy material needed for the demonstration of HHV-6 were included in the study. Follow-up data of 5 years were analyzed. The patient population has been described in detail previously [11]. Primary immunosuppression consisted of cyclosporine A, azathioprine and steroids. No anti-viral CMV prophylaxis was given postoperatively. The diagnosis of CMV infection was based on the standard CMV pp65 antigenemia test [15] and rapid shell vial cultures from blood and urine. Specimens for detection of CMV were obtained only, when clinical signs of CMV infection were suspected (fever, unexplained increase of serum creatinine, leukopenia, thrombocytopenia, hepatopathy, gastroenteritis, pneumonia).

The biopsy material was snap-frozen, and 3–4 micron thick sections were cut, acetone-fixed, and stored at −20 °C until used. CMV and HHV-6 antigens were demonstrated in kidney allograft biopsies using immunoperoxidase staining and monoclonal antibodies against CMV pp65 (Biotest, Dreieich, Germany) and HHV-6B (Chemicon Inc., Temecula, CA, USA) as described by Lautenschlager *et al.* [8]. All the biopsies used for the detection of CMV and HHV-6 were taken after confirming previous CMV infection and were either 6-month protocol biopsies or biopsies taken for clinical indications (deterioration of graft function). The biopsy histopathology was described according to Banff '97 [16] guidelines. As no extra biopsy or blood samples were taken for the purpose of this retrospective study, approval of the ethics committee was not required.

All data are expressed as mean ± 1 standard deviation, unless otherwise indicated. Statistical significances between the groups were measured by the nonparametric Mann–Whitney's *U*-test and Fisher's exact test, using SPSS statistical software (version 12.0.1.; SPSS Inc, Chicago, IL, USA).

## Results

During the 5-year follow up, one patient died and two patients returned to dialysis. CMV infection was diagnosed mean 47 ± 27 days after transplantation by positive pp65-antigenemia in all except one patient, in whom CMV was detected by viral culture from urine. CMV infections were treated with standard 2 weeks course of intravenous ganciclovir. Two patients, who demonstrated low level antigenemia which subsided during follow up, did not receive ganciclovir treatment. Other CMV infections were successfully treated, and no virus could be detected from urine or blood thereafter, or at the time of the biopsy in any of the patients.

Biopsies used for the detection of the viruses were taken mean 327 ± 364 after transplantation (range 30–1484). HHV-6 antigens were demonstrated in biopsies of 7/22 patients 18–1330 days after symptomatic CMV infection (mean 215 ± 346). HHV-6 antigens were detected in infiltrating leukocytes in the transplant of six patients, and in tubular epithelial cells in two patients (Fig. 1). Patients with positive and negative HHV-6 findings are further characterized in Table 1. Of the biopsies used for the detection of HHV-6 antigen, 13 were 6-month protocol biopsies and nine were biopsies taken for deterioration of graft function. Of the biopsies with a positive HHV-6 finding 4/7 were protocol biopsies, and of the biopsies with no HHV-6, 9/15 were protocol biopsies. Patients with a positive HHV-6 finding in the graft had a

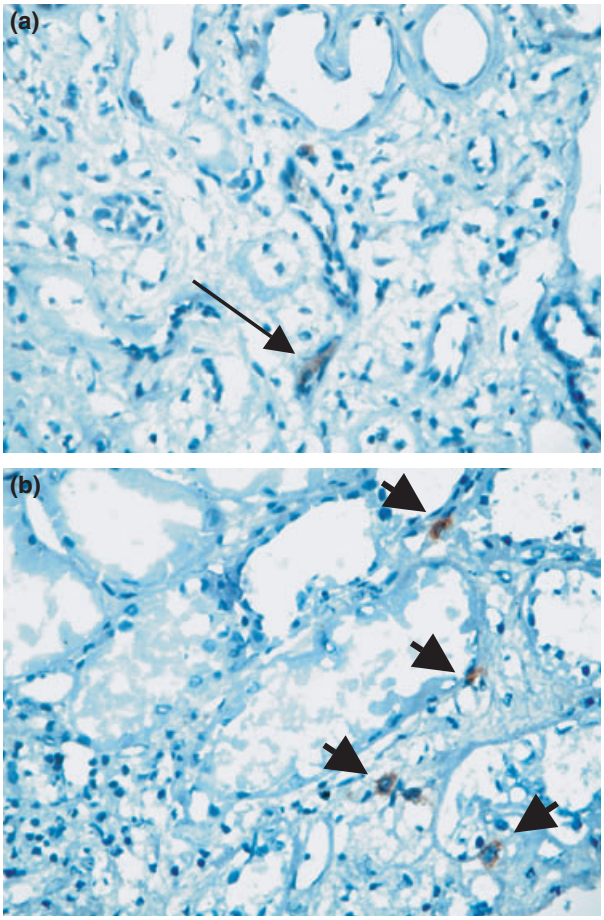


**Figure 1** HHV-6B antigen positive (a) tubular epithelial cells (long arrow) and (b) mononuclear inflammatory cells (short arrows) demonstrated by immunoperoxidase staning in frozen sections of kidney allograft biopsies. Original magnification 400×.

 

**Table 1.** Characterisation of the study patients.

| | HHV-6 positive in the graft ($n$ = 7) | HHV-6 negative in the graft ($n$ = 15) |
|---|---|---|
| Recipient age | 46 ± 7 | 54 ± 9 |
| Donor age | 34 ± 16 | 40 ± 16 |
| Time of biopsy for the demontration of HHV-6 (days post-transplantation) | 287 ± 341 | 373 ± 395 |
| Onset of first CMV infection (days post-transplantation) | 51 ± 23 | 45 ± 29 |
| Mean n:o of CMV infection episodes (range) | 1.6 (1–3) | 1.7 (1–5) |
| HLA-mismatch (A, B, and DR) | 2.1 ± 1.2 | 2.4 ± 0.6 |
| Acute rejection (yes/no) | 6/1 | 7/8 |
| Serum creatinine (μmol/l) at 5 years post-transplantation | 128 ± 43 | 259 ± 275 |

All data are expressed as mean ± SD.
All differences are nonsignificant.

slightly higher frequency of previous acute rejection episodes, but a positive HHV-6 finding was not associated with increased histopathologic changes in the biopsies (data not shown) or reduced renal function at 5 years after transplantation compared to patients with no intragraft HHV-6 (Table 1). The patients with negative HHV-6 finding showed a trend towards decreased renal function attributable to three patients with graft failure, but the difference was not significant. CMV antigens were found in the biopsies of seven patients. Only 1/7 of these biopsies demonstrated also HHV-6 antigens, whereas CMV antigens were found in 6/15 of the biopsies with no HHV-6. Persistent CMV antigens were demonstrated in the biopsies mean 93 ± 74 after last positive CMV finding from blood or urine. No correlation existed between CMV antigenemia levels and persistent intragraft CMV, or between CMV antigenemia levels and intragraft HHV-6 (data not shown).

Duration of i.v. ganciclovir treatment of first CMV infection episode did not differ between patients with intragraft HHV-6 and patients with no HHV-6 findings (data not shown). None of the patients received oral ganciclovir prophylaxis after intravenous treatment. Several CMV infection episodes developed in eight patients (range 2–4). Altogether 3/7 patients with a positive HHV-6 finding and 5/15 patients with no HHV-6 suffered from several CMV infection episodes. One reactivation in both groups was not treated with i.v. ganciclovir. These two patients demonstrated low level antigenemia, which subsided during follow-up. Other patients with reactivations received multiple courses of i.v. ganciclovir (total mean 19 days in HHV-6 positive patients and total 43 days in HHV-6 negative patients). One patient with no HHV-6 or CMV findings in the graft received a total 125 days of intravenous ganciclovir (four episodes) after transplantation because of persistent viremia.

## Discussion

Human herpesvirus-6 antigens were commonly demonstrated in renal biopsies of kidney allograft recipients with previous CMV infections. Although HHV-6 reactivations after organ transplantation often coexist with CMV infection [1,2], the presence of intragraft HHV-6 was not associated with CMV in our study, as only one patient demonstrated both viruses in the allograft biopsies.

A few other studies have also demonstrated HHV-6 antigens or DNA in kidney allograft biopsies [13,14]. In a study by Sebekova, in which herpesviruses were detected in the allograft biopsies by PCR, HHV-6 DNA was found also in late allograft biopsies [14]. A qualitative tissue PCR, however, may also detect latent or chromosomally integrated HHV-6 sequences [17], not necessarily an indicator of active infection, and histopathologic localization of the virus is not possible with this technique. Positive intragraft HHV-6 antigens, however, clearly represent protein synthesis and active infection. We found HHV-6B antigens mostly in infiltrating leukocytes, but also in tubular epithelial cells, in late allograft biopsies taken 30-1484 days after transplantation. These novel findings suggest that HHV-6 may persist in the allograft in a manner similar to its close relative CMV.

Although the persistence of CMV has been associated with detrimental effects to the kidney and liver allografts [11,18], the role of possible HHV-6 persistence in the allograft remains to be shown. In our relatively small size of study material, no increased histopathologic changes or reduced renal function could be demonstrated in patients with positive HHV-6 finding in the graft. A positive HHV-6 finding was, however, associated with slightly higher frequency of previous acute rejection episodes. Previous studies have found an association also between

Human herpesvirus-7 (HHV-7) and acute rejection [19]. HHV-7, a member of betaherpesviruses, often coexists with its close relatives of CMV and HHV-6 [2]. Analysis of HHV-7 was not included in this study, which may be a confounding factor in the association of HHV-6 and clinical outcome. Our study is also limited by the lack of blood analysis of HHV-6, which is attributable to the retrospective nature of this study. However, HHV-6 reactivations commonly occur during the first post-transplant month, and all except one of the positive HHV-6 findings in our study were demonstrated in biopsies taken 6 months or later after transplantation. The analysis of HHV-6 antigens in kidney allograft biopsies was limited only to patients with previous CMV symptomatic infections. Although certainly would be of interest, in this preliminary study we have not performed any studies on patients with asymptomatic CMV infection or low-risk patients with D-/R- constellation.

In conclusion, HHV-6 antigens were commonly found in late kidney allograft biopsies, suggesting that HHV-6 may persist in the kidney allograft. The nature and clinical relevance of this persistence remains to be elucidated.

## Authorship

IH: Primary researcher in this study. RL: Immunohistochemical analyses, virology. PK: Clinical nephrology, study design. IL: Virology, study design, head of the project.

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