Human Herpesvirus-6 Infection After Liver Transplantation

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A diagnosis of posttransplantation human herpesvirus-6 (HHV-6) infection was established for eight adult recipients among a liver transplantation patient population of 121. The diagnosis was based on serology and demonstration of HHV-6 specific antigens in liver biopsy specimens with use of monoclonal antibodies and immunoperoxidase staining. A significant graft dysfunction was recorded in association with serodiagnosis. HHV-6 early antigens, as well as HHV-6 variant B antigens, were detected retrospectively in all six available liver biopsy specimens. Histologic examination of biopsy specimens demonstrated acute rejection in 5 of the 8 patients, and 3 patients had portal lymphocyte infiltration. In five cases cytomegalovirus (CMV) infection was associated with HHV-6 infection; in four cases CMV antigens were also detected in the biopsy specimens. Two patients who had pure HHV-6 infection without CMV infection or rejection had significantly impaired graft function, with a positive antigen-detection test. Thus, HHV-6 may infect the liver allograft and cause graft dysfunction and may possibly be associated with rejection and/or CMV infection.

Human herpesvirus-6 (HHV-6) is one of the newest characterized members of the family of human herpesviruses [1–3]. Two major subgroups of the virus, variant A and variant B, have been identified [4]. Like other herpesviruses, HHV-6 can establish a latent or persistent infection that remains for the lifetime of the host and can reactivate during immunosuppression. The virus is closely related to human cytomegalovirus (CMV), and there are homologues between the two viral gene families [5, 6]. The clinical picture of HHV-6 infection varies from asymptomatic or a mild skin rash of exanthema subitum in infants to a febrile, severe or fatal disseminated disease in immunocompromised patients [3, 7]. In addition to disseminated infections, lymphadenopathy, encephalopathy, pneumonia, and hepatitis due to HHV-6 have also been described [8–12].

HHV-6 infections complicating the posttransplantation period have been reported to occur in bone marrow, kidney, and liver transplant recipients [9, 12–16]. In renal transplantation, the frequency of active HHV-6 infection has been reported to vary between 38% and 82% [13, 14], and asymptomatic seroconversions or increases in antibody titer are thought to be common. The presence of HHV-6 specific antigens in renal biopsy material has been demonstrated in association with other pathological conditions of kidney allografts, such as acute and chronic rejection or cyclosporine-related nephropathy [17, 18]. In particular, an association with allograft rejection, as well as with CMV infection, has been suggested [18, 19].

Herein we describe posttransplantation HHV-6 infection in eight adult liver transplant recipients. The diagnosis of HHV-6 was based on both serology (performed because of clinical suspicion of viral infection) and antigen detection in liver biopsy specimens (obtained because of graft dysfunction). The HHV-6 infections are described in relation to acute rejections and CMV infections.

Patients and Methods

Patients. During a 3-year period, since routine HHV-6 serology became available, HHV-6 infection was noted in eight adult liver transplant recipients among the patient population of 121 undergoing posttransplantation clinical follow-up at our center. The posttransplantation follow-up time for the patients varied from 1 to 9 years (median, 60 months). As basic immunosuppression, the patients received triple-drug therapy with various combinations of steroids, cyclosporine, and azathioprine. Acute rejections were treated with a high dose of methylprednisolone, and steroid-resistant rejections were further treated with OKT3. Diagnosis of rejections was based upon histologic findings at biopsy.

Laboratory diagnostics for viral infections were performed only in association with clinical suspicion. Severe CMV infections were treated with ganciclovir. Diagnosis of CMV infection was based on viral culture findings and detection of CMV-specific antigens in blood leukocytes or in biopsy material [20]. Serial liver function tests were performed to obtain the following values: serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyltranspeptidase (γ-GT), and bilirubin.

Diagnosis of HHV-6 infection. In this retrospective study, the diagnosis of HHV-6 infection was based upon both serology
and direct detection of HHV-6-specific antigens in the liver biopsy material. The serum specimens were obtained because of clinical suspicion of viral infection, and no regular follow-up of HHV-6 serology was performed for these patients. The liver allograft biopsies were performed because of graft dysfunction, to diagnose possible rejection or intragraft infections.

Histologic examination was performed on formalin-fixed and paraffin-embedded specimens of the same biopsy material. HHV-6 antigen detection was performed retrospectively on the available liver biopsy specimens obtained during the infection episode. Biopsy specimens of adult liver-transplant recipients with graft dysfunction but without serological evidence of HHV-6 infection were used as negative controls because no liver biopsy specimens from seronegative infants were available.

Serology. The HHV-6 antibody assay was performed by means of an indirect immunofluorescence test with use of HHV-6-infected HSB-2 cells (a continuous immature T-lymphoblastoid cell line) as antigens, as described in detail previously [21]. The acetone-fixed HHV-6-infected HSB-2 cells were incubated on object glass with patient serum samples at twofold dilutions of 1:10 to 1:160 for 30 minutes at 37°C and rinsed three times with PBS and once with distilled water. The second incubation was with fluorescein isothiocyanate (FITC)–conjugated goat antibody to human immunoglobulin (Kallestad, Austin, TX).

After being washed, the slides were examined with a fluorescence microscope. A serum known to contain HHV-6 antibody served as a positive control, and uninfected HSB-2 cells were used as a negative cell control. The titer was considered the reciprocal value of the last dilution sharing a virus-specific fluorescence. Altogether, 179 serum specimens obtained from the liver transplant recipients were analyzed. The serological diagnosis of HHV-6 infection was based on a fourfold increase in the antibody titer. In addition, an increase in titers to ≥1:160 was also considered diagnostic. Titters of ≥160 were considered significant because of their high association with recent infection [21], on the basis of an earlier serological screening survey of the Finnish adult population.

Detection of HHV-6 antigens in liver biopsy specimens. For HHV-6 antigen detection, the liver transplant core needle-biopsy material was snap-frozen, and sections (3–4 μm) were cut, acetone-fixed, and stored at −20°C until used. The presence of viral antigens was demonstrated by an indirect three-layer immunoperoxidase staining and a monoclonal antibody to an early HHV-6-specific antigen (MAB8533; Chemicon International, Temecula, CA) and another monoclonal antibody to HHV-6 variant B virion protein of 101 kD (MAB8535, Chemicon).

Before staining, the sections were treated with chloroform to eliminate nonspecific reactions due to endogenous peroxidase. A peroxidase-conjugated rabbit antibody to mouse immunoglobulin (Dako, Copenhaven) and a peroxidase-conjugated goat antibody to rabbit immunoglobulin (Tago, Burlingame, CA) were used as second and third antibodies. The reaction was revealed by 3-amino-9-ethyl carbazole solution containing hydrogen peroxide. Mayer’s hemalum was used for counterstaining. Biopsy specimens (n = 58) from the liver transplant recipients without serological evidence of HHV-6 infection were used as controls; 7 of these patients experienced acute rejection, and 11 were positive for CMV antigens but had no evidence of HHV-6 infection or rejection.

Liver biopsy histology. Liver allograft biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin wax. Four-micron-thick sections were prepared with a panel of five stains—hematoxylin-eosin, methyl green pyronin, Masson’s trichrome, diastase-PAS, and reticulin—to evaluate the histologic changes in the transplants. The histological diagnosis of acute hepatic allograft rejection was based on three main findings (which were also recommended recently [22]): mixed but predominantly mononuclear portal inflammation, bile duct inflammation and/or damage, and endothelitis of portal and/or terminal veins. At least two of these three alterations were required for the diagnosis.

Ethical considerations. All liver biopsy material investigated in this study was obtained because of clinical indications for histopathology.

Results

The appearance of HHV-6 infection, histologic findings in the liver biopsy related to HHV-6 serodiagnosis, CMV infections with antiviral treatments, and antirejection treatments are summarized in table 1. During a 3-year period, in a patient population of 121 adult liver transplant recipients undergoing clinical posttransplantation follow-up at our center, eight cases of HHV-6 infection were diagnosed by serology; infection appeared between 10 days and 5 years after liver transplantation. In four of the 8 cases, HHV-6 infection appeared within 2 months postoperatively. The other four HHV-6 infections were diagnosed late in the recipients’ follow-up course. A significant elevation of serum ALT (range, 182–1,550 IU), ALP (629–1,875 IU), γ-GT (618–1,770 μmol/L), and bilirubin (32–360 μmol/L) values was recorded in association with the serological diagnosis. Five patients also had fever.

HHV-6 early antigens were detected in all six available liver biopsy specimens obtained during the episode of graft dysfunction, which was closely concomitant with the serological diagnosis of HHV-6 infection. The HHV-6 antigens were located in scattered mononuclear leukocytes infiltrating the graft, mainly in the portal fields (figure 1). On the basis of their morphology, most antigen-positive cells were lymphocytes. In all six cases, HHV-6 variant B–positive leukocytes were also detected in the graft. HHV-6 antigens were not seen in the parenchymal cells of the liver. HHV-6 antigens were not detected in 39 liver biopsy specimens used as controls and obtained because of graft dysfunction from 19 patients without serological evidence of HHV-6 infection.
Table 1. The appearance of human herpesvirus-6 (HHV-6) infection, the day of serodiagnosis, antibody titers, detection of HHV-6 antigens in the graft, histologic findings related to HHV-6-infection serodiagnosis, antiviral treatments for cytomagalovirus (CMV) infection, and antirejection treatments in eight patients after liver transplantation.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Serology</th>
<th>Liver specimen histology</th>
<th>Febrile temperature (°C)</th>
<th>Histologic findings</th>
<th>CMV in blood</th>
<th>CMV in liver</th>
<th>Ganciclovir</th>
<th>Rejection treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,930 (160 to &gt;40)]</td>
<td>ND</td>
<td>39</td>
<td>Moderate acute rejection</td>
<td>. . . . . . .</td>
<td>1,930–1,934: MP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1,091 (640 to &gt;320)]</td>
<td>1,090</td>
<td>. . . . . . . . . . . . . .</td>
<td>Lymphocytic infiltration, parenchymal foci</td>
<td>. . . . . . .</td>
<td>. . . . . . . . . . . . . .</td>
<td></td>
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</tr>
<tr>
<td>3</td>
<td>29 (1,280 to &gt;320)]</td>
<td>ND</td>
<td>40</td>
<td>Lymphocytic infiltration</td>
<td>22</td>
<td>23–48</td>
<td>11–39: MP-OKT3-ATG</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>462 (10 to &gt;160)</td>
<td>462</td>
<td>40</td>
<td>Mild acute rejection</td>
<td>474</td>
<td>462–501</td>
<td>462–484: MP-OKT3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>263 (160 to &gt;1,260)</td>
<td>261</td>
<td>. . . . . . . . . . . . . .</td>
<td>Lymphocytic infiltration, parenchymal foci</td>
<td>. . . . . . .</td>
<td>. . . . . . . . . . . . . .</td>
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</tr>
<tr>
<td>6</td>
<td>10 (40 to &gt;640)</td>
<td>10</td>
<td>. . . . . . . . . . . . . .</td>
<td>Mild acute rejection</td>
<td>40</td>
<td>45–66</td>
<td>10–14: MP</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>35 (10 to &gt;640)</td>
<td>35</td>
<td>40</td>
<td>Resolving acute rejection, parenchymal foci</td>
<td>39</td>
<td>36–60</td>
<td>10–12: MP-OKT3</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>57 (40 to &gt;160)</td>
<td>57</td>
<td>38</td>
<td>Mild acute rejection, parenchymal foci</td>
<td>48</td>
<td>48–67</td>
<td>21–32: MP-OKT3</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. ATG = antithymocyte globulin; MP = methylprednisolone; ND = not determined (biopsy specimen not available).

* Titers 1–4 weeks later in brackets.

Biopsy histologic findings, obtained because of impaired graft function in association with HHV-6 infection, demonstrated signs of acute rejection in five patients. The other three biopsy specimens had mild to moderate portal inflammatory infiltration with lymphocytic predominance, but the criteria of rejection were not fulfilled. In four of the eight biopsy specimens, parenchymal small foci of inflammatory cells were also recorded. Six patients received high doses of steroids as antirejection treatment (with OKT3 or antithymocyte globulin in four cases) during the period of HHV-6-associated graft dysfunction.

In five of the eight cases, CMV antigenemia was associated with HHV-6 infection. In four patients CMV antigens were also demonstrated in the liver biopsy specimens, indicating graft affection. Five patients were treated with ganciclovir because of clinical CMV disease. Two patients (patients 2 and 5) who had a pure HHV-6 infection 9 months and 3 years, respectively, after transplantation.

Figure 1. Lymphocytes positive for HHV-6 antigen in liver allografts were demonstrated by indirect immunoperoxidase staining of frozen sections. HHV-6-positivity of the immunoperoxidase preparations is indicated by the brown staining of the cytoplasm of a few lymphoid cells (arrows) in the cellular infiltrate of the portal area (a: original magnification, ×100) and in a closer view of an HHV-6-positive lymphocyte (arrow) in the portal infiltrate (b: original magnification, ×400).
**Figure 2.** Serum levels of alanine aminotransferase (S-ALAT; IU), alkaline phosphatase (S-ALP; IU), γ-glutamyltransferase (S-γ-GT; μmol/L), and bilirubin (S-BIL; μmol/L) associated with human herpesvirus-6 (HHV-6) infection (a pure HHV-6 infection diagnosed 263 days after transplantation in patient 5).

HHV-6 infection, without CMV infection or rejection, had significant graft dysfunction (figure 2). HHV-6 antigens were also detected in the grafts. The graft histology demonstrated a mild to moderate, nonspecific portal inflammatory infiltration with lymphocytic predominance. In addition, inflammatory foci with lymphocytes and neutrophils were seen in the parenchyma. Increases in serum values of ALT, ALP, γ-GT, and bilirubin lasted a couple of months, but the values stabilized thereafter (figure 2). The level of HHV-6 antibodies increased slowly up to titers of 640–1,280 and remained high for several months. These patients were not treated with ganciclovir, but one of them received aciclovir for a few days, without any response in terms of liver function.

All patients who had an episode of HHV-6 infection are alive, but three patients (patients 3, 5, and 7) had to undergo retransplantation 7–36 months after transplantation because of chronic rejection and vanishing bile duct syndrome. Two of the patients have also had episodes of acute rejection and CMV infection. One patient (patient 5) had pure HHV-6 infection without CMV infection or acute rejection. This patient had persistently high HHV-6 titers, and antigen was detected in several liver biopsy specimens and in the explanted graft at retransplantation 30 months later.

**Discussion**

HHV-6 infection has been reported to occur in a small number of kidney transplant recipients [12–16], and only a few sporadic reports concerning liver transplant recipients have been published [16, 18, 19, 23]. It has also been shown that reactivations or reinfections in immunosuppressed individuals, such as with other viruses of the herpesvirus family (e.g., CMV), are quite common [24]. Even in normal children, the frequency of HHV-6 reactivations was reported to be as high as 16% over a period of 1–2 years in a prospective study [25].

Among our 121 adult liver transplant recipients, eight cases of serologically diagnosed HHV-6 infection were recorded, and only two cases were pure HHV-6 infection not associated with rejections or CMV infection reactivations. However, our study was retrospective, and the material represents only those cases diagnosed because of the evidence of graft dysfunction or clinical symptoms. The number of diagnosed reactivations would probably be much higher if the patients were regularly monitored for HHV-6 infection.

Our patients were all adults with reinfection or reactivation of HHV-6 infection and rather mild clinical signs or symptoms. None of our cases of HHV-6 infection was considered to be hepatitis at histology, as described previously in a case report of a primary infection [18]. Other investigators have reported that the presence of HHV-6 DNA can be detected by PCR in the blood of liver transplant recipients without any significant clinical symptoms at a frequency of 28% [23]. In general, it is possible that if the patient already has a diagnosed rejection and/or CMV infection explaining clinical symptoms such as fever or graft dysfunction, additional tests to detect HHV-6-
infection reactivations are not usually considered. On the other hand, in those cases of unexplained graft dysfunction, we should be more active in obtaining specimens to diagnose a possible HHV-6 infection reactivation.

As both HHV-6-specific early antigens and variant B antigens were demonstrated in the graft, it seemed that all the diagnosed HHV-6 infections in our liver transplant recipients were actually caused by the variant B virus. The variant B virus is more prevalent than the variant A and possibly associated with a different disease [24]. The HHV-6 subgroup B is responsible for most of the cases of primary infections documented in early childhood and etiologically associated with exanthema subitum [26].

According to our results, HHV-6 infection can be detected in the liver graft. In kidney allografts, HHV-6-specific antigens have been found in infiltrating cells and in the epithelial cells of the distal tubuli [14, 15]. We could not find HHV-6 antigens in the parenchymal structures of the liver, and the virus-positive cells were graft-infiltrating leukocytes, mainly lymphocytes. Although infected parenchymal cells were not detected, liver dysfunction was associated with the serodiagnosis and the presence of HHV-6 in the graft and was probably due to infection of the graft itself. To determine whether the virus was of donor or recipient origin, isolation of the strains and further gene sequence analysis would be needed.

For decades, the viruses of the herpesvirus family, especially CMV, have been suggested to be associated with rejection or even to trigger it. We and other investigators have recorded an association between chronic liver allograft rejection and persistent CMV infection [27, 28]. Whether HHV-6, as a close relative of CMV, may trigger the rejection cascade is not yet proven. In the publications concerning renal transplantation, an association between HHV-6 and allograft rejection has been recorded [14, 15]. Acute rejections were significantly more frequent in recipients with HHV-6 infections [14], and in biopsy specimens obtained during allograft rejection, HHV-6 antigen was common [15].

The involvement of the viral infection in rejection is possible because immunologic virus-host interactions have also been documented, and HHV-6 infection has been shown to induce the release of IFN-α and inflammatory cytokines, such as TNF-α and IL-1β [29, 30]. In our study, HHV-6 infection was in most cases detected in association with acute rejection and was detected in all three cases involving retransplantation due to chronic rejection.

For five of our liver transplant recipients, antirejection treatment preceded the HHV-6 diagnosis. However, we do not know whether the infection had already started before rejection, was concomitant with rejection, or occurred after rejection because of increased immunosuppression. Determining the exact timing of infection would require frequent monitoring of the virus or viral antigens in the blood or in the graft.

PCR techniques are already used to diagnose HHV-6 infection [23, 25, 31]. In adult transplant recipients, who are usually HHV-6 seropositive, PCR techniques to demonstrate viral DNA do not necessarily prove that there is an active infection (reactivation), but demonstration of mRNA by PCR would be very helpful in determining the exact timing of infection and investigating the association between HHV-6 and allograft rejection.

An association between CMV and HHV-6 infections has been noted in kidney transplant recipients [15, 16]. Among our liver transplant recipients who developed HHV-6 infection, CMV antigenemia was closely associated with diagnosis of HHV-6 infection in five cases, and in four of those cases CMV was also found in the graft. Probably both of these herpesviruses, which are close relatives and have genomic homologues, can also reactivate in similar situations, e.g., with increased immunosuppression during rejection episodes. Among our patients, the combination of rejection and CMV and HHV-6 infection was recorded in five cases. Whether both herpesviruses triggered acute rejection or were activated due to rejection or immunosuppression remains unproven, as does whether CMV and HHV-6 somehow activate mutually, which was suggested in a previous study of renal transplantation [16].

Those patients with pure HHV-6 infection also had lymphocytic infiltration of the graft, which was demonstrated in biopsy histology. Various degrees of lymphocytosis have been described to be associated with other viral infections as well, especially those with CMV and hepatitis C virus [32]. This type of nonspecific lymphocytic infiltration, which does not fulfill the criteria of rejection, may be due just to the immunoresponse against the virus but can make the differential diagnosis difficult in borderline cases of acute rejection. One of our patients was treated for rejection due to graft dysfunction, although the biopsy histology was inconclusive. Furthermore, in two patients with HHV-6 and CMV infections, acute rejection was suspected only from biopsy histologic findings, and antirejection therapy was administered with a good response.

We do not know yet whether HHV-6 (or CMV)—associated inflammation or other histopathologic changes may be falsely interpreted as borderline or mild rejection, in spite of using the criteria of the International Working Party [22], if the patient does not develop a clear picture of hepatitis.

In summary, HHV-6 may be reactivated and cause clinical problems, at least graft dysfunction after liver transplantation. HHV-6 antigens are detectable in the liver allograft during the infection episode. HHV-6 reactivations are often associated with rejections and CMV infection. HHV-6 antigen positivity is associated with lymphocyte infiltration of the graft, and the HHV-6-infected cells are located within the cellular infiltrate of the portal areas.

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References