Seroconversion to Human Herpesvirus 6 following Liver Transplantation Is a Marker of Cytomegalovirus Disease

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Human herpesvirus 6 (HHV-6) infection is common after transplantation; HHV-6 is known to interact with other viruses and induce immunosuppression. Whether HHV-6 plays a role in the occurrence of cytomegalovirus (CMV) infection after transplantation was investigated. In a cohort of 247 liver transplant recipients, HHV-6 seroconversion was identified as a significant risk factor for development of symptomatic CMV infection (P < .001), including CMV organ involvement (P < .001), even in the presence of the other significant risk factors: D/R CMV serologic status (P < .001) or use of OKT3 after transplantation (P = .002). Subgroup analysis indicated that HHV-6 seroconversion was significantly associated with symptomatic CMV infection in the D/R but not in the D+/R- CMV serologic group (P < .001 and P = .11, respectively). These results indicate that HHV-6 seroconversion is a marker for CMV disease after transplantation and suggest that additional studies using more sensitive diagnostic techniques are warranted to determine the relationship between HHV-6 and CMV infection after transplantation.

Human herpesvirus 6 (HHV-6) is a cytopathic lymphotropic virus with characteristic biologic effects, antigenic features, and endonuclease restriction patterns that distinguish it from other members of the herpesvirus family [1]. In vitro, cell tropism is most marked for T lymphocytes but has also been noted for B lymphocytes, monocytes, macrophages, glial cells, and fibroblasts [2]. Seroprevalence is almost universal at 2 years of age, and antibody is detected in 85%–100% of adults [3]. Primary infection with HHV-6 causes exanthem subitum (roseola infantum) in infants [4], while in older children and adults, infection may produce an infectious mononucleosis-like syndrome [5]. HHV-6 infection of peripheral blood mononuclear cells is marked by immunosuppression, with reduced cellular proliferation and interleukin-2 synthesis [6]. In addition, coinfections with both HHV-6 and other viruses, especially cytomegalovirus (CMV), are described, particularly in cases of pneumonitis after bone marrow transplantation [7]. Like other members of the herpesvirus family, HHV-6 may be cultured or cause clinical syndromes in immunocompromised hosts, including transplant recipients [8, 9]. While the estimated rate of detection of HHV-6 following organ transplantation ranges between 14% and 82% in different types of transplant recipients [10–12], its clinical impact after organ transplantation is unclear. Febrile illness with cutaneous rash, fulminant hepatitis, and interstitial pneumonitis have all been described [13, 14], as well as an association with increased graft rejection [9, 12]. In addition, an interrelationship between CMV and HHV-6 infection has been suggested by an increased frequency of rising IgG titers to HHV-6 following CMV infection [15].

After orthotopic liver transplantation, CMV is a frequent infectious complication leading to significant morbidity and mortality [16]. Prophylaxis and preemptive treatment with antiviral agents are two strategies aimed at reducing CMV infection [17] and require the identification of epidemiologic, clinical, or laboratory markers identifying the transplant recipient at increased risk of the development of symptomatic CMV infection. The epidemiologic risk factors thought to influence the shift from asymptomatic to symptomatic CMV infection after solid organ transplantation include belonging to the donor-positive/recipient-negative (D+/R-) CMV serologic status, as well as the use of OKT3, especially in the D+/R+ CMV serogroup [18, 19]. However, in this latter group, additional unidentified risk factors appear to be present to account for symptomatic CMV infection.

On the basis of special features of HHV-6 outlined above and the fact that HHV-6 infection temporarily precedes symptomatic CMV infection after solid organ transplantation [20, 21], we have investigated whether evidence of HHV-6 infection detected by serology is a significant risk factor for the development of symptomatic CMV infection after liver transplantation. Using a time-dependent multivariate analysis, we evaluated whether the serologic response to HHV-6 3 months after orthotopic liver transplantation was a risk factor for symptomatic CMV infection in a cohort of 247 transplant recipients.

Methods

Patients. A total of 247 first-time orthotopic liver transplantation recipients were studied. Patients who underwent retransplanta-
tion within the first 90 days were analyzed up to the time of retransplantation only. Mean age at the time of transplantation was 47.5 years, and 50.2% were men. Etiologies leading to liver transplantation were primary sclerosing cholangitis (68 patients), primary biliary cirrhosis (47), hepatitis C (18), hepatitis B (10), α1-antitrypsin deficiency (9), alcoholic liver disease (24), cryptogenic cirrhosis (25), autoimmune chronic active hepatitis (7), fulminant hepatic failure (9), tumors (10), and miscellaneous causes (20). CMV serostatus was as follows: donor positive/recipient positive (D+/R+), 105 patients (43%); D+/R−, 38 patients (15%); D−/R+, 76 patients (31%); and D−/R−, 26 patients (11%). In 2 patients, CMV serologic data were not available.

Transplant recipients belonging to the CMV D+/R− serogroup received CMV-seronegative blood products (for at least the initial 24 units). During the period in which these patients were transplanted, 117 patients entered into a study comparing two regimens of CMV prophylaxis. Fifty-five patients received intravenous ganciclovir (5 mg/kg twice a day) for 2 weeks followed by oral acyclovir (800 mg four times a day) for 14 weeks, and 62 received oral acyclovir (800 mg four times a day) for 16 weeks.

Immunosuppressive drug regimens consisted of prednisone in combination with cyclosporin and azathioprine or FK506, as previously described [22]. Biopsy-proven cellular rejection episodes were treated with intravenous methylprednisolone followed by OKT3 administration in the steroid-resistant cases as previously described [19].

HHV-6 antibody determination. Frozen (−70°C) stored serum obtained before transplantation and at 3 months after transplantation (mean, 98.7 days; median, 90) was analyzed for IgM and IgG anti–HHV-6 antibodies by an indirect immunofluorescence assay [1]. Samples with high levels of polyclonal immunoglobulin were pretreated with Zorba solution (Zeus Scientific, Raritan, NJ) to remove nonspecific IgG. Briefly, test slides containing cord blood mononuclear cells infected with HHV-6 subgroup B, strain Z-29 (Bion Enterprises, Park Ridge, IL) were reacted with different dilutions of the patient sera; specific antibodies were detected with fluorescent tagged anti-human IgM or IgG and read using a fluorescence microscope [3]. The results of the IgG serologies were expressed as the highest serial dilution at which fluorescence was detected. A titer of <1:10 was used as the cutoff to determine a negative result for HHV-6 IgG. Serologic conversion was defined as the presence of IgG or IgM positive for CMV before transplantation or by a >4-fold rise in IgG antibodies to HHV-6 after transplant with or without a concomitant positive IgM serology.

Seroconversion was defined as either primary seroconversion (the conversion of a pretransplant seronegative result for HHV-6 IgG to a posttransplant seropositive result) or as secondary seroconversion (a >4-fold rise in HHV-6 IgG with or without a concomitant positive IgM result). A panel of serially obtained sera from 19 liver transplant recipients was studied to determine evidence of cross-reactivity between anti–HHV-6 and -CMV antibodies. Only 5 of 12 samples in which seroconversion occurred to either CMV or HHV-6 had a simultaneous rise in antibodies to the other virus. In addition, sera from 19 patients were initially discordant for HHV-6 and CMV serostatus. These results support the specificity of anti–HHV-6 antibodies and their non–cross-reactivity with anti-CMV antibodies.

CMV serology, viral cultures, and definitions. Methods used for CMV detection have been previously described [23, 24]. Blood samples for CMV cultures were prospectively collected at weekly intervals for the first 2 months and at 3 months after transplantation. In addition, fluid or tissue specimens were obtained to detect CMV whenever CMV infection was clinically suspected. CMV IgG titers were determined by anti-complement immunofluorescence and IgM titers by indirect immunofluorescence.

CMV infection was defined as the isolation of CMV from any body fluid. CMV infection was deemed asymptomatic if infection occurred in the absence of clinical signs or symptoms and without CMV related laboratory abnormalities. Symptomatic CMV infection included CMV viral syndrome (fever, malaise, or arthralgias with associated leukopenia or thrombocytopenia) or organ invasion: pneumonia (parenchymal infiltrate on chest roentgenogram, hypoxia, and lack of other microbial pathogens) or gastrointestinal disease, including CMV hepatitis. Those definitions have been previously described [25].

Statistics. The risk of symptomatic CMV infection and CMV with organ involvement in the first 90 days after transplantation was estimated by use of the Kaplan-Meier method [26]. The relationship between the end points of symptomatic CMV infection and of CMV with organ involvement with HHV-6 infection and with known risk factors for the development of CMV infection—D/R CMV serostatus, fulminant hepatic failure before transplantation, pretransplant prothrombin time in the upper quartile, total units of red blood cells transfused in the upper quartile, total units of platelets transfused intraoperatively in the upper quartile, total units of fresh frozen plasma transfused intraoperatively in the upper quartile, and total of cryoprecipitate transfused intraoperatively in the upper quartile [27]—were compared in a univariate analysis using the log rank test. In addition, the following postoperative CMV risk factors were analyzed by time-dependent Cox modeling: OKT3 use postoperatively, hepatic artery thrombosis, and vanishing bile duct syndrome. In addition, the Cox model was used to assess HHV-6 status by multivariate analysis while adjusting one at a time for the other risk factors, namely D+/R− CMV serostatus and OKT3 use, previously found to be significantly associated with symptomatic CMV infection and CMV with organ involvement [28].

Results

Demographic data. The demographic characteristics of the study population as a whole or subdivided by HHV-6 seroconversion are shown in table 1. Age, sex, reason for liver transplantation, immunosuppression used, and antiviral prophylaxis were similar for all 3 groups. HHV-6 seroconversion (primary and secondary) was more frequent in the CMV D+/R− subgroup, 23 (60.5%) of 38, than in the CMV D+/R+ group, 19 (18%) of 105.

HHV-6 seroconversion and CMV events after transplantation. Thirty-three patients (13.4%) were seronegative for HHV-6 before transplantation. Twenty of them (61%) experienced primary HHV-6 seroconversion. Forty (19%) of 214 patients seropositive for HHV-6 before transplant developed secondary HHV-6 seroconversion. This yielded a total of 60 (24.3%) of 247 patients with evidence of HHV-6 seroconversion. IgM positive for HHV-6 was detected in 22 (9%) of 247 patients: 3 (15%) of 20 with primary HHV-6 seroconversion,
We examined the association between symptomatic CMV infection and HHV-6 infection in the CMV D⁺/R⁺ and D⁺/R⁻ serologic subgroups. As shown in table 3, the CMV D⁺/R⁺ subgroup with HHV-6 seroconversion had a higher incidence of symptomatic CMV infection than did the CMV D⁺/R⁺ subgroup without HHV-6 seroconversion (53% vs. 16%, P < .001).

Figure 1. Percentage of transplant recipients who developed symptomatic CMV infection over first 90 days after transplantation by HHV-6 serostatus.
pendent of the two previously identified risk factors: D+/R- CMV serostatus or OKT3 use. HHV-6 seroconversion as a marker of symptomatic CMV infection is most significant in the CMV D+/R- subgroup, in which the presence of the above-identified risk factors is insufficient to fully predict the risk of symptomatic CMV infection after transplantation.

Our data suggest that HHV-6 infection is a risk factor predisposing the patient to severe CMV infection; however, this is only implied from the use of serologic markers. Because serology is a suboptimal diagnostic method for the detection of herpesvirus infections in transplant recipients, results from this study suggest the need to study in further detail whether such findings are confirmed when more sensitive techniques (shell vial culture or polymerase chain reaction amplification of the HHV-6 genome) are applied to this study population. This will establish whether the association between HHV-6 seroconversion and CMV disease is due to HHV-6 infection or whether the seroconversion to HHV-6 is a marker of a particular state of immunologic status that predisposes to more severe CMV infection.

It is possible that HHV-6 seroconversion may be a better prognostic marker than more sensitive diagnostic techniques. For example, HHV-6 seroconversion could have been generated in response to a specific HHV-6 strain or to a high virus load present in tissues or body compartments, not detected by applying sensitive diagnostic techniques to peripheral blood samples. Alternatively, a genuine cross-reaction with a specific CMV protein that is not detected by conventional CMV serologic techniques may be present. If this were the case, the presence of these antibodies detected as anti–HHV-6 antibodies would be a marker of an immune response with an important role in CMV pathogenesis leading to invasive infection. Last, and through an initial evaluation (table 1), we have excluded cross-reactivity between CMV and HHV-6 antibodies that could have been present due to the homology between the gB of both viruses [29, 30]. Also, unlike acute Epstein-Barr virus infection, during which IgG antibody responses to other viruses occur with associated rises to both latent (herpesviruses) and nonlatent viruses (measles virus), the rises seen to both HHV-6 and CMV appear to occur in the absence of associated antibody rises [15, 29].

**Discussion**

This study indicates that HHV-6 seroconversion after liver transplantation is a marker of increased risk of symptomatic CMV infection in the first 90 days after transplantation, independent of the two previously identified risk factors: D+/R- CMV serostatus or OKT3 use. HHV-6 seroconversion as a marker of symptomatic CMV infection is most significant in the CMV D+/R- subgroup, in which the presence of the above-identified risk factors is insufficient to fully predict the risk of symptomatic CMV infection after transplantation.

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Our understanding of the mechanisms of potential HHV-6 interaction with CMV is hindered by the limited data on HHV-6 infection following orthotopic liver transplantation. Support for the concept of specific interactions between HHV-6 and CMV and their direct or indirect impact exist [13, 19]. HHV-6 infection is associated with more severe clinical symptoms if concomitant CMV infection is present [21], and serologically proven CMV infections are described in association with HHV-6 reactivations in solid organ transplant recipients [15]. Dual serologic responses to both HHV-6 and CMV are also described in immunocompetent persons [5, 29]. HHV-6 is a lymphotropic virus with particular tropism for the CD4+ lymphocyte, resulting in immunosuppression [6]. The recent finding that CMV gH and gL glycoproteins can form heterologous complexes with HHV-6 gH and gL glycoproteins raises a possible mechanism of interaction during cell coinfection [31]. The timing of HHV-6 infection at 2–4 weeks after transplant and before CMV infection occurs (6–12 weeks after transplant) would allow for interaction to occur [20, 21]. Alternatively, there may not be a direct interaction. HHV-6 seroconversion may be a marker of infection with as-yet-unidentified herpesvirus, which may interact with CMV and whose antibodies might be cross-reactive with those of HHV-6. The mechanism may also involve HHV-7, as it has been shown that HHV-7 infection after renal transplantation is a risk factor for symptomatic CMV infection [32].

Our data show that patients are often seronegative for both CMV and HHV-6 before transplant, and these persons develop severe primary CMV infections associated with primary HHV-6 infections. Infection with HHV-6 after transplantation may modify the course of CMV reactivation and make symptomatic infection, especially with organ involvement, more likely. Risk factors for HHV-6 infection after transplantation are based on anecdotal reports. A liver transplant recipient seronegative for HHV-6 before transplant developed HHV-6 infection after receiving a seropositive donor liver, while a renal transplant recipient developed HHV-6 infection after OKT3 use [33, 34]. As these risk factors are similar to those for CMV infection, coinfection may be facilitated.

Transfused blood products are a potential mechanism by which herpesviruses such as CMV may be transmitted after transplantation [35]. The same may be true of HHV-6. Allogeneic bone marrow transplant and orthotopic liver transplant recipients are both susceptible to HHV-6 infection after transplantation, and both transplant groups require large volumes of blood products [21, 36, 37]. Intravenous immunoglobulin transfusions may be a mechanism for passive transfer of immunoglobulin, giving rise to false-positive serologic results for HHV-6 IgG, as has been suggested in the case of allogeneic bone marrow transplant recipients [36]. The transfusion of blood products could also in theory lead to the passive transmission of HHV-6 IgG. However, liver transplant recipients receive the majority of their transfusions intraoperatively, and transfused antibodies are unlikely to be detected 90 days later. Similarly, none of our patients received intravenous immunoglobulin as CMV prophylaxis, so transfused antibody from that source was not possible. However, the possibility that the virus itself is transmitted in blood products remains and should be investigated in future studies.

Therefore, HHV-6 seroconversion may simply reflect a mode of infection common to both CMV and HHV-6 without interaction of the viruses. Ganciclovir use resolved the clinical syndrome associated with OKT3-associated HHV-6 infection and was also used successfully to treat a liver transplant recipient with febrile dermatosis and encephalopathy [34, 38]. As ganciclovir, but not acyclovir, shows antiviral activity against
HHV-6, its use prophylactically may decrease the incidence of HHV-6 infection [39].

In summary, HHV-6 seroconversion after transplantation is associated with symptomatic CMV infection, particularly in the D+/R- subgroup, in which this risk marker may modify CMV reactivation adversely, leading to more severe disease. Further studies are needed to explore this relationship and should use sensitive markers of infection, such as polymerase chain reaction amplification and culture techniques, to enable prompt diagnosis of HHV-6 infection. Once established, the standardized use of the test should enable surveillance for the early diagnosis of HHV-6 infection. This would enable the use of preemptive treatment strategies for CMV in those D+/R- persons with HHV-6 infection.

References