Human Herpesvirus 6 Reactivation Is Associated with Cytomegalovirus Infection and Syndromes in Kidney Transplant Recipients at Risk for Primary Cytomegalovirus Infection

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A potential association between human herpesvirus 6 (HHV-6) and cytomegalovirus (CMV) following kidney transplantation was explored by retrospectively testing serial serum specimens for HHV-6 IgG and IgM antibody. HHV-6 reactivation occurred in 35 (66%) of 53 transplant recipients. Fungal or parasitic opportunistic infections, graft rejection or loss, and mortality were not associated with HHV-6 reactivation. HHV-6 reactivation was associated with primary CMV infection ($P = .001$) and CMV syndrome ($P = .003$) and with trends for CMV-related hepatitis ($P = .095$), CMV-related neutropenia ($P = .104$), and serious CMV disease ($P = .085$). After controlling for CMV immune globulin (CMVIG) prophylaxis, the association between HHV-6 reactivation and primary CMV infection and syndrome remained significant ($P = .002$ and 0.006, respectively). The reduction in CMV syndrome among those receiving CMVIG prophylaxis remained significant ($P = .007$) after controlling for HHV-6 reactivation. HHV-6 reactivation in kidney transplant recipients at risk for primary CMV infection is associated with CMV infection and CMV-related disease, and these effects are independent of CMVIG prophylaxis.

Infection with human herpesvirus 6 (HHV-6) is common following solid organ transplantation, affecting 24%–82% of transplant recipients [1–5]. Recently, a potential interaction between HHV-6 and cytomegalovirus (CMV) following solid organ transplantation has been noted. In solid organ transplantation, patients coinfected with both HHV-6 and CMV may have more severe clinical manifestations of infection [2, 6]. In addition, HHV-6 infection has been associated with symptomatic CMV infection [1] and graft rejection, with or without CMV infection, following liver transplantation [7].

We explored a potential interaction between HHV-6 and primary CMV infection in solid organ transplantation, which previously demonstrated a significant reduction in CMV disease using CMV immune globulin (CMVIG) prophylaxis in kidney transplant recipients at risk for primary CMV infection [8]. To determine the effect of HHV-6 infection on the occurrence and severity of CMV infection in these kidney transplant recipients, we analyzed stored serial serum specimens for HHV-6 infection.

Methods

Patient population. We retrospectively evaluated serial serum specimens from a cohort of 53 kidney transplant recipients. These patients were originally involved in a prospective, randomized, multicenter, controlled trial evaluating the efficacy of CMVIG prophylaxis to prevent primary CMV infection and disease in renal transplant recipients [8]. Fifty-nine patients were involved in the original trial, but adequate serum samples were not longer available for 6 patients. To be eligible for the original trial, all recipients had to be susceptible to CMV, as indicated by a reciprocal CMV antibody titer $<8$ by indirect hemagglutination assay. In addition, all donors were required to have antibody to CMV, as evidenced by reciprocal titer of $\geq8$. Patients received their kidney transplantation between October 1982 and January 1986 at one of the participating centers [8].

Twenty patients received CMVIG via the protocol previously described [8]. None of the 53 patients received any other form of antiviral prophylaxis. Patients were followed weekly for the first 8 weeks after transplantation, monthly for the next 6 months, and again at 1 year after transplantation. Each patient’s clinical care was individualized by the transplantation team at the participating institution according to that medical center’s protocol. Baseline laboratory studies were done at enrollment, weekly for 2 months following transplantation, when special problems arose, and at all subsequent follow-up visits as noted above. Documentation of CMV infection by serology and/or virus isolation was done as previously described [8]. Clinical data with regard to CMV-asso-
Serum samples obtained within 72 h after transplantation, weekly for 8 weeks, monthly for an additional 6 months, and at 1 year were stored at −20°C and available for analysis. Same-day postinfusion serum samples were also available from all CMVIG-treated patients. Donor specimens and pretransplant sera were not available for this analysis. Serologic testing for HHV-6 IgG antibody was done with a commercially available ELISA (Advanced Biotechnologies, Columbia, MD). For initial screening, specimens were diluted 1:20. Untreated control patients had serum specimens tested for IgG antibody within 72 h of transplant and on weeks 1, 6, 12, 32, and 52 after transplant. Twenty-five of 33 control patients had sera available at or beyond week 32. For CMVIG-treated patients, we tested specimens within 72 h of transplant and at weeks 1, 4, 6, 12, 32, and 52; samples from weeks 4, 6, and 12 had HHV-6 IgG antibody determined on both pre- and postinfusion sera. All 20 CMVIG-treated patients had sera available at or beyond week 32. After this initial screening, the 2 specimens with the lowest and highest optical density from each patient were diluted from 1:50 to 1:12,800 and reanalyzed for HHV-6 IgG antibody by the same ELISA. Paired samples from a given patient were always tested simultaneously on the same microtiter plate. When a dilution was done on a specimen obtained the same day a patient received CMVIG, a preinfusion serum specimen was always used.

Serum specimens obtained weekly for the first 8 weeks after transplantation and at 12 weeks were tested for HHV-6 IgM antibody by a commercially available indirect immunofluorescent assay (Advanced Biotechnologies, Columbia, MD). Samples were diluted at 1:80, higher than the manufacturer’s recommendation of 1:20, to improve the specificity of the assay. A fluorescence intensity of ≥2+ was considered positive. Fluorescence intensity was determined by reviewers blinded as to the specimen source and CMV infection or antibody result.

**Definitions.** Infection with HHV-6 was defined as either a ≥4-fold increase in IgG antibody or the presence of IgM antibody. CMV infection was defined as seroconversion, isolation of virus from any body fluid or tissue, or detection of virus in tissue specimens [8].

CMV syndrome was defined as unexplained fever for at least 3 days in association with one of the following: pneumonitis without other causes, leukopenia (<3000 white blood cells/mm³) on ≥3 consecutive days after withdrawal of azathioprine or ganciclovir, an elevated serum alanine aminotransferase level (≥2.5 times normal) in the absence of other viral causes, or atypical lymphocytosis (>20% of peripheral white blood cells). Thrombocytopenia was defined as <100,000 platelets/mm³. Serious CMV disease was defined as disease occurring in association with at least one of the following: leukopenia (<3000 white blood cells/mm³), fungal or parasitic superinfection, CMV pneumonia, retinitis, or central nervous system involvement.

**Statistical analysis.** Univariate analyses were done by χ² testing. Multivariate analysis were done with logistic regression modeling controlling for HHV-6 infection and CMVIG prophylaxis. All statistical analyses were done using the SAS system for Windows, version 6.12 (SAS Institute, Cary, NC).

### Results

**HHV-6 serology and infection.** HHV-6 reactivation was demonstrated in 35 (66%) of 53 patients following kidney transplantation. Evidence for HHV-6 reactivation was determined as follows: ≥4-fold rise in HHV-6 IgG antibody and presence of HHV-6 IgM antibody in 15 patients, ≥4-fold rise in HHV-

### Table 1. Characteristics and non-CMV-related outcomes in kidney transplant recipients with and without HHV-6 reactivation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HHV-6 reactivation (n = 35)</th>
<th>No HHV-6 reactivation (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>30.4 ± 13.2</td>
<td>32.6 ± 15.5</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22 (63)</td>
<td>10 (56)</td>
</tr>
<tr>
<td>Female</td>
<td>13 (37)</td>
<td>8 (44)</td>
</tr>
<tr>
<td>Transplant type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Living related</td>
<td>17 (49)</td>
<td>9 (50)</td>
</tr>
<tr>
<td>Cadaver</td>
<td>18 (51)</td>
<td>9 (50)</td>
</tr>
<tr>
<td>Initial immunosuppression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prednisone/azathioprine</td>
<td>7 (20)</td>
<td>5 (28)</td>
</tr>
<tr>
<td>Prednisone/cyclosporine</td>
<td>19 (54)</td>
<td>5 (28)</td>
</tr>
<tr>
<td>Antilymphocyte serum (and above)</td>
<td>6 (17)</td>
<td>3 (17)</td>
</tr>
<tr>
<td>Prednisone/cyclosporine/azathioprine</td>
<td>3 (9)</td>
<td>5 (28)</td>
</tr>
<tr>
<td>CMVIG prophylaxis</td>
<td>12 (34)</td>
<td>8 (44)</td>
</tr>
<tr>
<td>Fungal or parasitic opportunistic infection</td>
<td>4 (11)</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Graft rejection</td>
<td>19 (54)</td>
<td>8 (44)</td>
</tr>
<tr>
<td>Rejection therapy</td>
<td>11 (31)</td>
<td>6 (33)</td>
</tr>
<tr>
<td>Any ATG or T12 monoclonal antibody</td>
<td>7 (20)</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Graft loss</td>
<td>8 (23)</td>
<td>3 (17)</td>
</tr>
<tr>
<td>Mortality</td>
<td>2 (6)</td>
<td>2 (11)</td>
</tr>
</tbody>
</table>

NOTE. Age is given as mean ± SD; all other data are no. (%). ATG, antithymocyte globulin; CMVIG, CMV immune globulin. None of the differences were statistically significant.

* Any rejection therapy ≤2 weeks after transplant.
6 IgG antibody without HHV-6 IgM antibody in 15 patients, and presence of IgM antibody without a ≥4-fold rise in IgG antibody in 5 patients. All serum specimens tested from control (average of 5 specimens per patient) and CMVIG-treated (average of 10 specimens per patient) transplant recipients were positive for HHV-6 IgG antibody at a dilution of 1:20.

In the subset of 20 patients with detectable HHV-6 IgM antibody, the median time to detection after transplant was 47 days (range, 11–107). The time to 4-fold rise in HHV-6 IgG antibody cannot be accurately reported, since only selected samples after transplant were screened for IgG antibody. Examining the overall antibody positivity to HHV-6 and CMV among the 33 placebo patients only, to avoid possible antibody detection secondary to CMVIG administration, we found that 100% of the 169 samples had detectable HHV-6 antibody; CMV antibody was detected in only 38% (64/169) of the samples. Clearly, antibody was being detected that could not be attributed to CMV.

Patient characteristics and non–CMV-related outcomes. Patient characteristics and selected non–CMV-related outcomes in patients with and without HHV-6 reactivation are shown in table 1. Patient age, sex, type of transplant, initial immunosuppressive regimen, or administration of CMVIG prophylaxis had no significant effect on rates of HHV-6 reactivation. Fungal or parasitic opportunistic infections, graft rejection or loss, and mortality were not associated with HHV-6 reactivation. We examined the effect of therapy for rejection on HHV-6 reactivation by assuming that any rejection that occurred ≤2 weeks following transplantation would precede HHV-6 reactivation. Rejection therapy, including the subgroup receiving antithymocyte globulin or T12 monoclonal antibody, was not associated with HHV-6 reactivation.

Influence of HHV-6 reactivation on CMV infection and associated diseases. Primary CMV infection occurred in 41 (77%) of 53 kidney transplant recipients. The effect of HHV-6 reactivation on the occurrence of primary CMV infection was significant: 32 (91%) of 35 HHV-6-infected patients had primary CMV infection, compared with 9 (50%) of 18 patients without HHV-6 reactivation (P = .001; table 2). In addition, HHV-6 reactivation had a significant effect on the development of CMV syndrome: 21 (60%) of 35 patients with HHV-6 reactivation developed CMV syndrome, as opposed to 3 (17%) of 16 patients without HHV-6 reactivation (P = .003). The presence of HHV-6 reactivation was also associated with a trend toward the occurrence of other CMV-related syndromes, such as hepatitis (46% vs. 22%; P = .095), neutropenia (31% vs. 11%; P = .104), and serious CMV disease (40% vs. 17%; P = .085). Although the number of patients with CMV pneumonitis, thrombocytopenia, or retinitis was small, HHV-6 reactivation had no association with the rates of these CMV-associated diseases.

After controlling for the effect of CMVIG prophylaxis by logistic regression analysis, the association between HHV-6 reactivation and primary CMV infection (odds ratio [OR] = 10.39; 95% confidence interval [CI] = 2.30–47.01; P = .002) and CMV syndrome (OR = 8.70; 95% CI = 1.89–40.12; P = .006) remained significant. Conversely, the reduction in CMV syndrome among those who received CMVIG prophylaxis, previously demonstrated in these patients, remained statistically significant (OR = 0.14; 95% CI = 0.03–0.58; P = .007) after controlling for the presence of HHV-6 reactivation.

Discussion

Our investigation indicates that HHV-6 reactivation is common following kidney transplantation—occurring in 66% of patients. In addition, HHV-6 reactivation is associated with CMV infection and CMV syndrome in kidney transplant recipients at risk for primary CMV infection. A trend toward the development of serious CMV disease, hepatitis, and neutropenia was also observed. The effect of HHV-6 reactivation on the development of CMV syndrome was independent of the administration of CMVIG prophylaxis. HHV-6 reactivation had no effect on graft rejection or patient mortality.

There is accumulating evidence to suggest an association between HHV-6 and CMV infection following organ transplantation. HHV-6 has been documented to have immunomodulating effects in vitro. HHV-6 can induce production of  

**Table 2.** CMV infection and CMV-associated diseases in kidney transplant recipients with and without HHV-6 reactivation.

| Variable                  | HHV-6 reactivation (n = 35) | No HHV-6 reactivation (n = 18) | P
|---------------------------|----------------------------|--------------------------------|---
| Primary CMV infection     | 32 (91)                    | 9 (50)                         | .001
| CMV syndrome             | 21 (60)                    | 3 (17)                         | .003
| CMV serious disease      | 14 (40)                    | 3 (17)                         | .085
| Other CMV-associated diseases |                     |                                |    
| Hepatitis                 | 16 (46)                    | 4 (22)                         | .095
| Pneumonitis               | 4 (11)                     | 1 (6)                          | NS
| Neutropenia               | 11 (31)                    | 2 (11)                         | .104
| Thrombocytopenia          | 7 (20)                     | 3 (17)                         | NS
| Retinitis                 | 2 (6)                      | 0                              | NS

**NOTE.** Data are no. (%). NS, not significant.
interleukin-1β and tumor necrosis factor-α [9], suppress T lymphocyte function due to reduced interleukin-2 synthesis [10], and suppress bone marrow by inducing interferon-α [11]. In vivo data have also documented a bone marrow-suppressive effect of HHV-6 infection [12–14]. Such immunomodulating effects could create a state of immunosuppression, predisposing the transplant recipient to opportunistic infection with CMV or other infectious pathogens similar to those seen with the herpes-group viruses. Moreover, limited data on the timing of infection with HHV-6 after transplant suggests that HHV-6 infection occurs earlier than CMV infection. Isolation of HHV-6 after solid organ transplant or bone marrow occurs within the first 4 weeks [2], in contrast to CMV infection, which typically occurs 4–16 weeks after transplantation [8]. An early HHV-6–induced state of immunosuppression would allow for the development of more severe CMV-associated disease.

Other clinical studies have demonstrated an interaction between HHV-6 and CMV after transplantation. In allogeneic bone marrow transplantation, active CMV infection was shown to be associated with HHV-6 isolation [15]. Herbein et al. [2] demonstrated that severe clinical symptoms, usually attributable to CMV infection, occurred in solid organ transplant recipients with HHV-6 infection only if patients were coinfected with CMV. Chapman et al. [6] reported that the combination of HHV-6 and CMV infection after renal or pancreas transplantation was more strongly associated with a clinical syndrome consistent with CMV disease than with either virus alone. Dockrell et al. [1] showed that HHV-6 seroconversion was a marker for CMV disease after liver transplantation independent of other risk factors for symptomatic CMV infection. A recent study in liver transplant recipients by Lautenschlager et al. [7] described 8 cases of HHV-6 reactivation and associated graft dysfunction. In 6 of these cases, evidence for concurrent CMV infection was also noted. Thus, our results confirm those of other investigators by documenting an association between HHV-6 and CMV infection and related disease and extend these observations by showing that this association is independent of CMVIG prophylaxis.

In conclusion, we have shown that HHV-6 reactivation in renal transplant recipients at risk for primary CMV infection is associated with CMV infection and disease and that this effect is independent of CMVIG prophylaxis. In addition, the reduction in CMV syndrome that occurs with CMVIG prophylaxis in this transplant population is not explained by HHV-6 reactivation. Since this study deals with an earlier era in transplantation, future studies should explore the effects of current immunosuppressive regimens, antirejection treatments, and antiviral prophylaxis on the development of HHV-6 infection. In addition, our study used serology only; therefore, examination of serial serum specimens with new viral diagnostic modalities, such as an antigen-capture assay or quantitative polymerase chain reaction for HHV-6, should be used to increase the sensitivity of detection of HHV-6 reactivation and develop a greater understanding of the temporal relationship between HHV-6 and CMV.

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References