The Prevalence of Human Herpesvirus–7 in Renal Transplant Recipients Is Unaffected by Oral or Intravenous Ganciclovir

Daniel C. Brennan,1 Gregory A. Storch,1,2 Gary G. Singer,1 Linda Lee,1,a Jose Rueda,1 and Mark A. Schnitzler1

The purpose of this study was to compare the prevalence of human herpesvirus (HHV–7) and cytomegalovirus (CMV) viremia and the effects of oral and intravenous (iv) ganciclovir in renal transplant recipients at risk for CMV. Stored lysates from peripheral blood leukocytes from 92 patients, who had been previously analyzed for CMV viremia by polymerase chain reaction (PCR) for 12 weeks after transplantation, were analyzed for HHV–7 viremia. Baseline and peak prevalences of HHV–7 viremia were 22% and 54%, respectively (P < .0001). Eighty-two (89%) of 92 patients had at least 1 positive PCR for HHV–7. Oral ganciclovir and treatment with iv ganciclovir had no effect on the prevalence of HHV–7 viremia. In contrast, CMV was almost completely suppressed in patients who received oral ganciclovir, and when present, CMV responded to iv therapy. These results indicate that HHV–7 is resistant to ganciclovir at levels that were effective for prevention and treatment of CMV.

The significance of human herpesvirus (HHV–7) in renal transplant recipients is unknown. HHV–7 is a betaherpesvirus that is biologically similar to HHV–6 and to cytomegalovirus (CMV). The clinical disease caused by HHV–7 is not clearly elucidated. HHV–7 has been found to cause asymptomatic infection in childhood and has been implicated in exanthem subitum–like illness (roseola), meningoencephalitis, febrile convulsions, chronic fatigue, and pityriasis rosea [1–5]. HHV–7 is postulated to be a cofactor for human immunodeficiency virus, and it may also be a cofactor for CMV [6, 7]. In one study that compared infection with CMV alone and coinfection with CMV and HHV–7, the relative risk (RR) of progression to CMV disease following coinfection with CMV and HHV–7 was high (RR, 3.5; 95% confidence interval [CI], 1.1–11.6) [7]. At least 1 review has suggested that HHV–7 is suppressed by intravenous (iv) ganciclovir [8]. It is unknown whether oral ganciclovir can achieve adequate serum levels for suppression or treatment of HHV–7. The aims of the present study were to compare the prevalence of HHV–7 and CMV viremia over time in renal transplant recipients who were at risk for CMV disease and to compare the sensitivity of HHV–7 and CMV viremia to oral and iv ganciclovir.

Materials and Methods

Study population. The patient population consisted of 92 adult renal transplant patients who were at risk for CMV and who had participated in 1 of 2 studies in which DNA was obtained weekly, for at least 12 weeks after transplantation, for purposes of analysis. All patients were adult renal transplant recipients at Washington University/Barnes Hospital, and all patients had a CMV serologic status of either donor (D)–positive (+)/recipient (R)–negative (−), D+/R−, or D−/R+. Patients had been randomized to receive either preemptive therapy or deferred therapy (study 1) and either prophylactic oral ganciclovir or deferred therapy [9, 10]. Patients randomized to receive “preemptive” iv ganciclovir were intravenously treated with ganciclovir, 5 mg/kg, every 12 h for 21 days based on a positive CMV polymerase chain reaction (PCR) result or a positive CMV culture, regardless of the presence or absence of symptoms. Patients randomized to the “deferred” group received ganciclovir only when a positive CMV PCR result correlated with symptoms of CMV disease [9]. Patients in the deferred group also received prophylactic oral acyclovir (200 mg) twice daily for herpes simplex virus prophylaxis for 12 weeks. Patients randomized to the prophylactic oral ganciclovir group received 1000 mg 3 times per day for 12 weeks [10]. Patients were instructed to take oral ganciclovir with food to increase bioavailability [11]. The immunosuppressive regimens for the subjects, procedures for collection of data, definitions of symptomatic CMV infection, and ganciclovir regimens used to treat symptomatic episodes were described previously [9, 10]. In brief, all but 1 patient received polyclonal anti-lymphocyte induction therapy with either horse antithymocyte
gamma globulin (Atgam, Pharmacia-UpJohn, Kalamazoo, MI) or rabbit antilymphocyte gamma globulin (Thymoglobulin; SangStat, Fresno, CA). Maintenance immunosuppression was accomplished with cyclosporine (Neoral; Novartis, East Hanover, NJ) or tacrolimus (Prograf; Fujisawa, Deerfield, IL); azathioprine or mycophenolate mofetil (CellCept; Roche Pharmaceuticals, Nutley, NJ); and prednisone. Symptomatic CMV infections were intravenously treated with ganciclovir (5 mg/kg) every 12 h for 21 days.

**Specimens.** In both studies, peripheral blood leukocytes had been collected from all patients on a weekly basis for ≥12 weeks after transplantation, and leukocytes were analyzed by qualitative PCR for the presence of CMV DNA. In the present study, lysates (stored at −70°C) from these patients were analyzed by PCR for HHV-7 DNA.

**PCR.** Qualitative detection of HHV-7 was performed by individuals who had been blinded to the clinical course of the patients. The PCR was performed on stored lysates (80 μL containing 1 million cell equivalents) of leukocytes, using a modification of the technique of Wilborn et al. [12]. The HHV-7 primer pair was published elsewhere by Berneman et al. [13]. Oligonucleotides were obtained from Oligos, Etc. (Wilsonville, OR). All samples from each individual patient were amplified in the same reaction (sequentially by date). Each reaction contained ≥1 positive control and ≥1 negative control. A reaction was considered valid only if the positive controls yielded a 186-bp band and if the negative control lacked a band at the same location. In brief, each PCR tube contained 70 pmol of each primer, 2.5 μL of dNTP (5 mM of each), 10 mM Tris-HCl (pH, 8.3), 50 mM KCl, 1.5 mM MgCl₂, 1.25 U of Taq, and 8.0 μL of template in a total volume of 50 μL. The template consisted of 8 μL of the stored DNA lysate (100,000 cell equivalents), high-purity water as a negative control, or a plasmid preparation containing 100 copies of amplicon as a positive control. The amplification reaction was initiated by hot start at 94°C for 3 min. Each cycle consisted of denaturation at 94°C for 30 s, primer annealing at 54°C for 30 s, and extension at 72°C for 30 s. Forty cycles were completed before 5 min of final extension at 72°C. All reactions were prepared under a laminar flow hood, and the investigators wore caps, gloves, and gowns to prevent contamination. All equipment was wiped down with 10% bleach solution and treated with ultraviolet light between reactions. PCR products were handled in a separate room for analysis and storage. The PCR assay had the capability to detect 1 plasmid copy per reaction.

**Controls.** A cell suspension of 1 million human cord-blood lymphocytes infected with HHV-7 strain SB was obtained from the American Type Culture Collection (Lot 1 WE, Rockville, MD), and this suspension was lysed. PCR was performed from a 1 : 10 dilution (10,000 leukocyte equivalents) to yield a 186-bp amplicon. As described by Wilborn et al. [12], we noted that the target amplification of 186 bp was digested by EcoRI to produce fragments of 126 and 60 bp. A plasmid preparation containing the target 186-bp amplicon was cloned with the TA Cloning Kit, version 2.0 (Invitrogen Corp., Carlsbad, CA). A purified preparation was created with the QIAGEN (Chatsworth, CA) plasmid purification protocol. The plasmids were suspended in Tris-HCl (10 mM; pH, 9.0). After quantification of the DNA, serial dilutions were made. The positive controls were plasmid preps of 100 genome copies of the amplicon suspended in Tris-HCl (10 mM; pH, 9.0).

**Detection.** Amplification products were separated by gel electrophoresis on 3% NuSieve agarose (FMC Corp., Rockland, ME) at 200 V, and amplification products were visualized by ethidium bromide staining. Each run contained a molecular-weight ladder (BioMarker Low; BioVentures, Murfreesboro, TN), a negative control, and a positive control lane for comparison with other lanes. Photographs of our results were obtained and stored using a video imaging system (Model IS 1000; Alpha Innotech Corp., San Leandro, CA).

**Determination of sensitivity.** The DNA in the plasmid preparations was quantified using the 4,6-diamidino-2-phenylindole method, per Kapuscinski and colleagues [14, 15]. From the initial very concentrated suspension of 8.42 μg of DNA/mL (1.86 × 10⁴ molecules/μL), serial dilutions were made at a separate laboratory to prevent widespread contamination. Inhibition of PCR was ruled out, and preservation of human DNA was confirmed by a separate reaction with either beta globulin or human leukocyte antigen–DQ primers. By use of the method described above, the detection limit was determined to be 1 genome copy per reaction. As a further control, the detection limit was determined both with and without a background leukocyte lysate of 100,000 cells, which were obtained from 2 healthy volunteers who had tested negative for HHV-7 PCR.

**Statistics.** Patient demographic information and baseline characteristics were summarized using descriptive statistics. For continuous variables, treatment groups were compared using the standard 2-sample t test. For highly skewed data, the Wilcoxon rank sum test was used. For binary categorical variables, comparisons were made with Fisher’s exact test. For multicaegorical variables, comparisons were made with χ² tests. Time-to-event analyses were based on the log rank test [16]. Event-free rates were calculated by the Kaplan-Meier method [17].

Daily viral prevalence curves were constructed by methods similar to Kaplan-Meier actuarial survival curves [17]. Patients were included in the analysis starting on the day of their first posttransplantation sample. The prevalence at week 1 (day 7) was chosen as the baseline starting point, because patients enrolled in the prophylactic oral ganciclovir versus the deferred therapy group for CMV study did not have samples obtained for DNA on day 0. A patient’s viral status (positive or negative) on days during which no sample was available was assumed to equal the result of the previous sample. Thus, a change in a patient’s status could be triggered only by known measured data. A patient was censored on the day following the day of his or her last sample and was
Figure 1. Daily prevalence of human herpesvirus (HHV)-7 and cytomegalovirus (CMV) viremia during the first 84 days after transplantation in all groups combined. The prevalence of patients receiving intravenous ganciclovir on each day is also shown. The baseline prevalence of HHV-7 was 22% (see text). The peak prevalence occurred at day 28 and measured 52%, which was significantly different from baseline ($P = .019$). The baseline prevalence of CMV was 0%, and the peak prevalence was 45% at day 42 ($P < .001$) compared with baseline.

Results

Demographics. The pretransplantation demographics of the groups are shown in table 1. Among the 92 patients, 26 were randomized to receive oral ganciclovir prophylaxis, 15 were randomized to receive preemptive therapy with iv ganciclovir upon detection of active CMV viremia, and 51 were randomized to receive deferred therapy for symptomatic CMV disease. There were no differences among the groups at baseline. Overall, 74% of the recipients received renal allografts from cadaveric donors, 18% of the recipients had diabetes as the cause of end-stage renal disease, 40% of recipients were African American, 64% of recipients were men, and the mean age of the recipients was 46 years.

Rejection. Overall, 10 (11%) of 92 patients experienced an acute rejection episode in the first 12 weeks after transplantation, as shown in table 1. There was no difference in the incidence of acute rejection among the groups ($P = .886$). There was also no difference in the agents used for treatment of rejection ($P = .436$). Two patients in the oral ganciclovir group were treated with steroid boluses, and 1 was treated with OKT3 (Monomurab; OrthoBiotech, Raritan, NJ). In the deferred group, 4 patients were treated with steroids and 2 with OKT3. In the preemptive group, 1 patient was treated with OKT3. There was no effect of the rejection treatment on the prevalence of HHV-7 ($P = 1.000$).

Overall prevalence of HHV-7 and CMV viremia and use of iv ganciclovir. The prevalence of HHV-7 and CMV viremia for all groups analyzed together can be seen in figure 1. The baseline prevalence of HHV-7 viremia at week 1 was 22%, and prevalence rose rapidly after transplantation, reaching 52% by day 28. The prevalence ranged between 42% and 54% over the next 56 days. The peak prevalence of HHV-7 viremia was 54% ($P < .0001$, vs. baseline) at day 70. During the first 84 days, 82 ($P < .0001$) of 92 patients had at least 1 positive PCR for HHV-7. No patients in any group had detectable CMV viremia at baseline (figure 1). The prevalence of CMV viremia rose more slowly after transplantation than it did for HHV-7, starting to rise at 14–21 days after transplantation and peaking at 45% on day 42 after transplantation ($P < .0001$). The prevalence ranged between 35% and 41% over the next 42 days. Excluding patients who received oral ganciclovir, 60 (91%) of 66 patients had evidence of CMV viremia at some time during the first 84 days after transplantation (see below). The rate of increase in the prevalence of CMV plateaued concurrently with increasing prevalence in the use of iv ganciclovir (figure 1).

Forty (43%) of the 92 patients received iv ganciclovir for CMV during the first 12 weeks after transplantation. No patients who received oral ganciclovir prophylaxis received iv ganciclovir during this time period (see below). Thus, excluding the oral ganciclovir group, 40 (61%) of 66 patients received iv ganciclovir.

Effect of oral ganciclovir on the prevalence of HHV-7 and
To investigate the effect of oral ganciclovir on the prevalence of HHV-7 and CMV viremia after transplantation, we analyzed the prevalence of these viruses in those patients who received oral ganciclovir compared with those who did not receive oral ganciclovir (figure 2). Oral ganciclovir had no demonstrable effect on the prevalence of HHV-7 viremia (figure 2, top). The peak prevalence of HHV-7 in the oral ganciclovir group was 73% on day 84. The peak prevalence of HHV-7 in those that did not receive oral ganciclovir was 53%. In contrast, oral ganciclovir had a dramatic effect on the prevalence of CMV (figure 2, bottom). Indeed, it almost completely prevented reactivation of CMV during the time of administration. In contrast, the peak prevalence of CMV in the group that did not receive oral ganciclovir was 62% (P < .001, vs. baseline).

Effect of iv ganciclovir on the prevalence of HHV-7 and CMV viremia. To compare the effects of iv ganciclovir on HHV-7 and CMV viremia, we determined the per-day prevalence of HHV-7 and CMV viremia relative to the time of initiation of iv ganciclovir administration for the patients who received iv courses of the drug (figure 3). Treatment with iv ganciclovir had no obvious effect on the prevalence of HHV-7 viremia during the administration of ganciclovir. The prevalence of HHV-7 was 32% the day prior to administration and 45% on the last day of iv ganciclovir treatment (P = .333). In contrast, the prevalence of CMV viremia was 89% at the time of the initiation of treatment with iv ganciclovir, and this value decreased to a low of 21% after 21 days of treatment (P < .0001).

Discussion

The present study demonstrated that the baseline prevalence of HHV-7 viremia was 22% and that the baseline prevalence of CMV viremia, as measured by PCR, was 0% at the time of transplantation in those at risk for CMV (donor, recipient, or both; seropositive for CMV). The higher prevalence of HHV-7 viremia may reflect a true increase or an increased sensitivity of the HHV-7 assay (compared with the CMV assay). Most patients (89%) had detectable HHV-7 viremia during at least 1 week of the first 12 weeks after transplantation. Excluding those who received oral ganciclovir (which prevents reactivation of CMV), 91% of at-risk patients developed CMV viremia during the first 12 weeks after transplantation. The prevalence of both HHV-7 and CMV viremia increased over the first 12 weeks after transplantation, although HHV-7 was reactivated more rapidly. These observations demonstrate that reactivation of both viruses is associated with transplantation and immunosuppression.

The use of prophylactic oral ganciclovir had no effect on HHV-7 viremia, but it almost completely prevented the reactivation of CMV. This was a surprising result, considering that
both viruses are betaherpesviruses and that their genomes are highly homologous.

The use of iv ganciclovir also had no effect on the prevalence of HHV-7 viremia. However, ganciclovir use was associated with a rapid decrease in the prevalence of CMV viremia. These data demonstrate that the lack of effect of oral ganciclovir on HHV-7 viremia was not simply a dose effect; these data also demonstrate that the levels of ganciclovir achieved with usual doses of iv ganciclovir are inadequate to control HHV-7 viremia.

Since the completion of our study, the sensitivity of HHV-7 to 4 classes of antiviral compounds has been studied in vitro [18]. These classes included (1) a pyrophosphate analogue, phosphonoformic acid (Foscarnet); (2) beta-guanine analogues (acyclovir, ganciclovir, and penciclovir); (3) cidofovir and several related compounds; and (4) a series of benzimidazole ribonucleosides. HHV-7 was found to be most sensitive to cidofovir and related compounds, with an effective inhibitory concentration (EC_{50}) of 3 \mu g/mL. HHV-7 was least sensitive to the beta-guanine analogues, with an EC_{50} of >7 \mu g/mL for ganciclovir and 12–128 \mu g/mL for acyclovir and penciclovir.

The mean IC_{50} to ganciclovir for most CMV isolates is 1–3 \mu g/mL [19–22]. Maximum iv concentrations (C_{max}) of ganciclovir at a 5 mg/kg dose are 3–7 \mu g/mL [21–23]. Oral ganciclovir may achieve a C_{max} of 1–2.5 \mu g/mL in transplant recipients, depending on the degree of renal insufficiency [20]. Thus, the in vivo findings of our study concur with those of the recently reported in vitro study [18] involving HHV-7 and those of the previous studies of CMV.

HHV-7 is increasingly being recognized as a potential pathogen in immunosuppressed patients. HHV-7 may be a cause of unexplained viral syndromes that fail to respond to ganciclovir therapy in transplant recipients. We have demonstrated that prophylactic oral ganciclovir did not prevent reactivation of HHV-7, and iv ganciclovir did not treat HHV-7 viremia. These results indicate that HHV-7 is resistant to ganciclovir at levels that were effective for prevention and treatment of CMV. Further study of the resistance of HHV-7 to ganciclovir may provide insights into the mechanism of action of ganciclovir resistance and sensitivity.

References