β-Herpesvirus (Human Cytomegalovirus and Human Herpesvirus 6) Reactivation in At-Risk Lung Transplant Recipients and in Human Immunodeficiency Virus–Infected Patients

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Human herpesvirus (HHV)-6 is a β-herpesvirus-like human cytomegalovirus (HCMV) with the potential to reactivate in immunocompromised persons. HHV-6 and HCMV were assessed in the peripheral blood leukocytes of 26 lung transplant recipients and of 37 human immunodeficiency virus (HIV)-infected patients receiving highly active antiretroviral therapy, to determine the degree of concordance between HHV-6 and HCMV reactivation in different biologic settings. In the lung transplant recipients (145 samples), HHV-6 was not detected, even though 44 (30%) of 145 samples were from 9 HCMV DNA–positive patients (13 episodes of HCMV pneumonitis). Among the HIV-infected patients (172 samples), HCMV DNA was detected in 29 (17%) of 172 samples from 10 patients (4 episodes of HCMV disease). HHV-6 DNA was detected in 2 HIV-infected patients who did not have HCMV detected at that time. These findings suggest that the pathobiologic control mechanisms for these 2 β-herpesviruses may be significantly different.
same samples that were assessed for HCMV. Because there are limited data regarding possible HHV-6 activity among LTRs, the role of HHV-6 in infecting LTRs who have a high incidence of HCMV disease and who are routinely given ganciclovir prophylaxis early after transplantation remains to be elucidated.

HIV-infected patients have a variable risk of opportunistic infections depending on disease severity, their use of prophylactic antimicrobial agents, and their response to HAART. HIV-infected patients with CD4 cell counts <100 × 10^6 cells/L were previously considered to be at high risk for HCMV reactivation prior to the routine use of HAART. The incidence of HCMV disease significantly declined with the introduction of HAART for HIV-infected patients and those with AIDS [7, 8]. In this study, we examined HHV-6 and HCMV loads in a cohort of patients with HIV or AIDS to assess the relationship between HCMV and HHV-6 reactivation.

The role of HHV-6 in a cohort of LTRs with a relatively high incidence of HCMV reactivation and pneumonitis and in at-risk patients with HIV or AIDS receiving HAART with a low risk of HCMV reactivation is particularly of interest, given the unique models of immunosuppression that these disease states provide. In addition, the recent concepts of biologic synergism and transactivation among related herpesviruses [23–25] and the potential relationship between β-herpesvirus reactivation and allograft rejection syndromes [26] and HIV disease progression [27, 28] strongly argue for the concurrent examination of β-herpesvirus reactivation. This examination in 2 immunosuppressed cohorts with varying incidence of HCMV disease offers important pathobiologic insights into β-herpesvirus reactivation.

Subjects and Methods

Subjects

LTRs: Alfred Hospital, a Monash University–affiliated teaching hospital in Melbourne, Australia, has a national heart and lung transplant service that has performed an average of 35–40 lung transplants each year over the last 5 years. Between June 1997 and August 1998, 46 lung transplants were performed. Seventeen LTRs were not studied because of prolonged follow-up was to occur in interstate centers. Of the remaining 29 LTRs, we monitored 26 with repeated blood tests and bronchoscopic sampling at the Alfred Hospital. All had monthly samples obtained during the first 6 months after transplantation, as described elsewhere [11]. Three patients were not studied because of prolonged immediate postoperative complications.

All patients were treated immediately after transplantation with a standard immunosuppressive regimen composed of cyclosporine, azathioprine, and prednisolone, as described elsewhere [11]. Ganciclovir prophylaxis was given to at-risk patients (donor [D] and/or recipient [R] seropositive for HCMV) at a dose of 5 mg/kg intravenously twice a day for 2 weeks, followed by 5 mg/kg 3 times a week for ≥8 weeks after transplantation. The prophylaxis was increased to 12 weeks if there was a primary HCMV mismatch (D/R-). The latter patient group also received HCMV hyperimmune globulin on days 1, 2, 3, 7, 14, 21, 28, and 35 after lung transplantation. HCMV pneumonitis was defined as a clinical HCMV syndrome in association with histopathologic evidence of HCMV inclusions in transbronchial biopsies. Bronchoscopy, bronchoalveolar lavage sampling, and transbronchial biopsies were performed as part of routine surveillance after lung transplantation at weeks 2, 4, 8, 12, and 26 and when clinically indicated. HCMV pneumonitis episodes were treated with a repeat course of full-dose ganciclovir (5 mg/kg twice daily for 2 weeks).

HIV-infected subjects. The Alfred Hospital is a major referral center for patients with HIV. Between August 1997 and June 1999, 37 HIV-infected patients who were at risk for HCMV reactivation (CD4 cell count <100 × 10^6 cells/L) who were HCMV antibody positive were randomly recruited from the outpatient HIV clinic and followed for 1 year. Most HIV-infected patients (92%) were receiving HAART for the duration of this study. Ophthalmologic assessments were done at 0, 3, 6, 9, and 12 months. HCMV disease in HIV-infected patients was defined as ophthalmologically proven HCMV retinitis or histopathologically proven disease in other tissues. After the diagnosis of HCMV disease, HIV-infected patients received specific anti-HCMV therapy, beginning with ganciclovir induction (10 mg/kg/day for 14–21 days given daily), followed by ganciclovir maintenance doses of 10 mg/kg 3 times a week.

Peripheral Blood Samples and Sample Preparation

Peripheral blood samples consisted of 6 mL of blood in EDTA vacuum tubes. Samples were processed within 24 h of collection. Peripheral blood leukocytes (PBL) were isolated from 3 mL of blood (in duplicate) by use of a dextran-based density gradient. After a series of lysis steps and washes, PBL pellets were resuspended in 0.5 mL of PBS, snap frozen in liquid nitrogen, and stored at −80°C.

DNA Extraction

We used the QiAamp blood kit (QIAGEN) to extract DNA from stored samples, as noted in the manufacturer’s instructions. An RNase step was incorporated into the procedure to remove any contaminating RNA species that could interfere with accurate measurements of DNA. Extracted DNA was resuspended in 100 µL of elution buffer and stored at −80°C. DNA purity and concentration were assessed by using capillary cuvette microsample spectrophotometry.

HCMV and HHV-6 PCR Assays

DNA extracted from PBL samples collected were tested for both HHV-6 and HCMV DNA. HCMV quantitative PCR was performed by use of a competitive HCMV PCR assay, as described elsewhere [11, 29]. We developed a similar assay for the quantitation of HHV-6 DNA.

HHV-6 PCR Assay

HHV-6 oligonucleotides. HHV-6–specific primers directed at a conserved region of the HHV-6 DNA polymerase gene (5′-CAGC-
CAGTCCCTTAAGTAGCA and 3′-AACAATAGACACGGAATAGGTA) were selected from HHV-6 genome strain U1102 (GenBank accession no. X83413), by use of Oligo 5.0 Primer Analysis Software (National Biosciences), to amplify a native 278-bp fragment. We used the software to select primers to ensure that there was no cross-reactivity for all other parts of the HHV-6 genome and all other HHVs. Primer pairs selected amplified both common strain HHV-6 variants A (strain GS [A\textsubscript{GS}]) and B (strain Z29 [B\textsubscript{Z29}]).

**Construction of HHV-6 competitor.** A 150-bp HHV-6 internal standard molecule was constructed by oligonucleotide synthesis (Auspex). This standard had a 128-bp deletion, compared with the native fragment, but had primer binding sites identical to the native fragment at both the 5′ and 3′ ends. The HHV-6 internal standard molecule was resuspended in sterile water and diluted to working concentrations with 50 μg/mL glycogen (Boehringer Mannheim Australia) in 10 mmol/L Tris-HCl (pH 7.5).

**HHV-6 competitive PCR.** Quantitative competitive PCR was done by using known amounts of competitor molecule. PCR reagents (Perkin Elmer) in a final 50-μL volume were as follows: 1 μg of sample DNA, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2 mM MgCl\textsubscript{2}, 200 μM nucleotides, 1 U of Taq gold DNA polymerase, and 0.2 μM each oligonucleotide primer. Master mixes were used whenever possible, and positive and negative controls were used. PCR amplification was conducted for 50 cycles with a preactivation step (94°C for 8 min), denaturation (94°C for 30 s), annealing (58°C for 30 s), and a final extension (72°C for 7 min) in a thermal cycler fitted with a heated lid (Perkin Elmer).

**Specificity of assay.** The HHV-6 primers produce 279-bp native fragment and 150-bp competitor molecules. To confirm identity of the PCR products, competitive PCR was performed by using a known HHV-6–positive isolate (provided by the Victorian Infectious Disease Reference Laboratory, Melbourne, Australia) (figure 1). HHV-6 variant specificity was assessed by using A\textsubscript{GS}-positive and B\textsubscript{Z29}-positive DNA (provided by Bill Rawlinson, Prince of Wales Hospital, New South Wales, Australia). Bands were identified by DNA sequencing. Eight single PCRs for each native HHV-6 and competitor fragment, respectively, were carried out. Reactions were pooled and purified directly by use of the QIAquick (QIAGEN) PCR purification kit. Purified PCR products were used for DNA sequencing. HHV-6–positive samples were identified by variant typing (HHV-6A and -6B) by Theo Sloots (Sir Albert Szentegi Virus Research Centre Royal children's Hospital, Queensland, Australia).

**Quantitation.** Amplified PCR products were analyzed in 4% agarose gels. We used a fluorescent scanner (FluorImager; Molecular Dynamics) to detect patient HHV-6–positive DNA (native HHV-6 DNA) and competitor fluorescent bands. The relative fluorescence of the 2 bands was quantitated with Image QuaNT software (Molecular Dynamics). Quantitation of native HHV-6 DNA was achieved when a ratio was measured between native and competitor bands after PCR with known amounts of competitor molecule. Typically, patient DNA samples were screened in duplicate with 1 μg of DNA and 100 copies of HHV-6 competitor molecule. When both native and competitor molecules were quantifiable, the ratio between them could be measured, and, thus, the input number of native molecules could be determined. DNA levels were reported as copies of DNA per micrograms of total DNA extracted. The detection limit of the HHV-6 assay was 10 copies/μg of total DNA extracted for both HHV-6 variants A and B.

**Validation of competitive PCR assay.** A change in ratio experiment was made, in which the concentration of native HHV-6 DNA template in the PCR was kept constant, but the amount of competitor internal standard was varied. This titration showed that the ratios closely followed the predicted line, indicating that both HHV-6 competitor and HHV-6 native products competed equally in the PCR (figure 2).

**Results**

**Patient Cohorts**

**LTRs.** In total, 26 LTRs were studied, and 145 samples were collected during the first 6 months after transplantation (mean, 6 samples/patient; range, 1–8 samples/patient). One patient had only a single sample collected, because of early death, and 2 patients had 8 samples collected because extra clinical reviews occurred in the period immediately after the first 6 months after transplantation. Of the LTRs, there were 11 men and 15 women (mean age, 41 years; range, 22–62 years). HCMV D/R serostatus of the patients was as follows: 10 D+/R-, 8 D+/R+, 6 D-/R+ and 2 D-/R-. HCMV pneumonitis was observed in 9 patients. There were 13 HCMV disease episodes, and 4 of the 9 patients experienced 2 HCMV disease episodes (table 1). Of the 9 patients in whom HCMV pneumonitis was observed, there were 4 HCMV D+/R+ and 3 D-/R- matches and 2 D-/R+ mismatches.

**HIV-infected patients.** In all, we studied 37 patients with HIV or AIDS with CD4 cell counts of <100 × 10\(^6\) cells/L. All...
were men. Their mean age was 43 years (range, 28–68 years). For 18 of the 37, all their CD4 cell counts were $<100 \times 10^3$ cells/L during the collection period (median time, 4 months). Nineteen patients had at least 1 CD4 cell count $>100 \times 10^3$ cells/L (median time, 4 months). Of these patients, 172 samples were collected over a 2-year period (mean, 5 samples/patient; range, 2–8 samples/patient). The variations in numbers of samples collected were due to both clinical review factors and logistic constraints during the follow-up period. All 37 patients were HCMV seropositive. HCMV disease occurred in 4 patients: 3 developed retinitis, and 1 developed colitis and esophagitis. There were a total of 6 episodes of disease; 2 patients experienced 2 episodes each (table 1).

HCMV Detection in Clinical Samples

**LTRs.** We previously published the HCMV DNA results and described their relationship to histopathologically proven HCMV pneumonitis in our cohort of LTRs [11]. In summary, HCMV DNA detection in patients at any time during the first 6 months after lung transplantation had a sensitivity of 100% and a specificity of 59% for HCMV pneumonitis (table 2). HCMV DNA in any sample taken over the study period had a sensitivity of 92% and a specificity of 76% for HCMV pneumonitis. HCMV DNA was detected in 12 of the 13 PBL samples taken at the time of HCMV pneumonitis. Most cases of HCMV pneumonitis (10 of 13 episodes) occurred 3–6 months after transplantation, and 6 of the 13 occurred 2–3 months after transplantation, immediately after the cessation of ganciclovir prophylaxis. Of interest, 1 LTR had resistance to ganciclovir and had persistent low HCMV detection after treatment with ganciclovir. This was confirmed by molecular testing for ganciclovir resistance in 5 sequential samples over a 4-month period. A mutation in the phosphotransferase UL97 gene was identified as the site of resistance.

**HIV-infected patients.** HCMV DNA was detected in 10 of 37 patients at any time point during the study period. Four of the 10 patients with detectable HCMV DNA had HCMV disease (sensitivity, 100%). HCMV DNA was not detected at any time in the remaining 27 patients, and none had disease (specificity, 82% for HCMV DNA detection; positive likelihood ratio, 5.5; table 3). In all, 172 samples from the 37 patients were collected during the study, and HCMV DNA was detected in 29 of samples. Seventeen samples were collected from patients with HCMV disease, and HCMV DNA was detected in all 17 (sensitivity, 100%). HCMV DNA was detected in 2 of the 6 episodes of HCMV disease at the time that HCMV disease was diagnosed. In the remaining 4 episodes, HCMV was detected either 2 weeks before or after disease diagnosis (but still within the disease episode period) because of clinical review constraints. Twelve of the 29 samples in which HCMV DNA was detected were from patients without HCMV disease. Of the 172 samples, no HCMV DNA was detected in 143 samples, all from patients without disease (specificity, 92%; positive likelihood ratio, 12.5; table 3).

HCMV levels in the 4 patients with HCMV disease were higher at episodes of disease and declined by $\geq 10$-fold when 3 of the 4 patients were receiving anti-HCMV therapy. In 1 patient, virus loads continued to increase rapidly, despite ganciclovir maintenance and change in anti-HCMV treatment to foscarnet induction doses. HCMV DNA levels reached 100,000 HCMV DNA copies/$\mu$g at the time of the second episode of HCMV retinitis. We suspected that this patient had ganciclovir resistance, but this was not confirmed with molecular testing. In the remaining 6 patients in whom HCMV was detected and who had no clinical evidence of HCMV disease, HCMV DNA was detected in relatively low amounts ($<220$ HCMV copies/$\mu$g). Of the 32 samples collected from these 6 patients, HCMV DNA was detected in 12 samples.

**HHV-6 PCR Assay**

We developed an HHV-6 competitive PCR assay with an internal control to quantitate HHV-6 DNA in a cohort of LTRs and HIV-infected patients. Competitive PCR with an HHV-6–

**Figure 2.** Test of native and competitor polymerase chain reaction (PCR) by titration of competitor. Human herpesvirus (HHV)-6 was titrated against a constant aliquot of HHV-6 native DNA, and PCR was done. Results are quantitation of the native:competitor ratios for HHV-6. *Sloped line,* line of prediction of 1.

**Table 1.** Patient cohort demographics. Comparative PCR with an HHV-6–

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lung transplant recipients</th>
<th>HIV-infected patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients ($n = 63$)</td>
<td>26</td>
<td>37</td>
</tr>
<tr>
<td>No. of samples ($n = 317$)</td>
<td>145</td>
<td>172</td>
</tr>
<tr>
<td>Age, mean years (range)</td>
<td>41 (22–62)</td>
<td>43 (28–68)</td>
</tr>
<tr>
<td>Sex, male:female</td>
<td>11:15</td>
<td>37:0</td>
</tr>
<tr>
<td>HCMV disease, no. of cases</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Total no. of episodes</td>
<td>13</td>
<td>6</td>
</tr>
</tbody>
</table>

**NOTE.** HCMV, human cytomegalovirus; HIV, human immunodeficiency virus.
HHV-6–positive samples were positive and HHV-6–negative patients with HIV. Virus loads in all time. Figure 3 shows a representative example of HHV-6–positive isolate and HHV-6A and the predicted size (278 bp). DNA sequencing confirmed identification of both the HHV-6–positive isolate and HHV-6A and -6B variants. We assessed cross-reactivity of HHV-6 primers for differentiation of both the HHV-6–positive isolate and HHV-6A and -6B variants. We assessed cross-reactivity of HHV-6 primers for HCMV by competitive PCR with known HCMV-positive and -6B variants. We assessed cross-reactivity of HHV-6 primers for HCMV by competitive PCR with known HCMV-positive and -negative samples and failed to amplify native HCMV DNA (data not shown). The HHV-6 primers selected did not cross-react with other herpesviruses. We assessed herpes simplex virus (HSV) types 1 and 2, varicella-zoster virus (VZV), Epstein-Barr virus, HCMV, HHV-7, and HHV-8 by use of Oligo 5.0 primer analysis software (National Biosciences).

**HHV-6 Detection in Clinical Samples**

**LTRs.** HHV-6 DNA (variants A and B) was not detected in any of the 145 samples from the 26 LTRs, including the 9 patients with histopathologically proven HCMV pneumonitis and high HCMV DNA loads in several samples during the first 6 months after lung transplantation (table 4).

**HIV-infected patients.** Of interest, only 2 (5.4%) of the 37 HIV-infected patients studied had HHV-6 DNA detected in PBL samples (table 5). Neither patient had any episode of HCMV disease, nor was HCMV DNA detected at any point in the study. HHV-6 DNA was detected in 5 of 6 samples from patient 1 over the collection time period and in 2 of 6 samples from patient 2. Of the 2 HIV-infected patients with HHV-6 detection, both had CD4 cell counts >100 × 10⁶ cells/L—one for the whole collection time and the other for half the collection time. Figure 3 shows a representative example of HHV-6–positive and HHV-6–negative patients with HIV. Virus loads in all HHV-6–positive samples were <200 copies/μg of total PBL DNA extracted. Although there was not a striking difference in HIV loads between HHV-6 DNA–positive (4.38 copies/μg of total DNA [range, 3.2–5.2 copies/μg of total DNA]) and negative (4.54 copies/μg of total DNA [range, 1.7–5.98 copies/μg of total DNA]) patient samples, there was a tendency for higher CD4 cell counts in the HHV-6 DNA–positive group (159 copies/μg of total DNA [range, 74–261 copies/μg of total DNA] vs. 57 copies/μg of total DNA [range, 2–375 copies/μg of total DNA], respectively).

Additional clinical information at the time that HHV-6 was detected in the 2 patients mentioned above is as follows: during the period in which patient 1 had an HHV-6 DNA–positive PBL sample, he had a decline in HIV RNA and an increase in CD4 cell count to 264 cells/μL in response to a change in anti-HIV therapy to stavudine, lamivudine, and indinavir. He had 2 episodes of shingles that responded to valaciclovir. The anti-HIV therapy of patient 2 was changed to saquinavir and ritonavir just before the follow-up period, in an attempt to maintain virologic control (a tendency toward >5,000 HIV copies/mL). He was HHV-6 DNA positive twice; the second time was associated with a febrile illness and rash in the right side of his face. Subsequent neurologic investigations at the latter time point revealed a normal computed tomography scan of the head and negative cerebrospinal fluid (CSF) PCR results for HSV, VZV, and CMV. His CSF showed 718,000 copies/mL of HIV RNA and peripheral HIV RNA of 30,000 copies/mL. He was empirically treated with valaciclovir and subsequently with foscarinet, with good clinical effect.

**Discussion**

HHV-6 was assessed in a cohort of LTRs with a relatively high prevalence of HCMV reactivation and disease and in HIV-infected patients with a relatively low prevalence of HCMV reactivation and disease. A quantitative assay for HHV-6 var-

<table>
<thead>
<tr>
<th>Patients (n = 26)</th>
<th>HCMV</th>
<th>No HCMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR detection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCMV pneumonitis</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>No HCMV pneumonitis</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 3. Polymerase chain reaction (PCR) detection of human cytomegalovirus (HCMV) in peripheral blood leukocytes (PBL) and HCMV pneumonitis among human immunodeficiency virus–infected patients.

<table>
<thead>
<tr>
<th>PCR detection of HCMV</th>
<th>HCMV disease</th>
<th>No HCMV disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n = 37)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>No</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>PBL samples (n = 172)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>No</td>
<td>0</td>
<td>143</td>
</tr>
</tbody>
</table>

* No. of HHV-6 DNA–positive samples detected by PCR, with or without HHV-6 DNA detected at any time point in the follow-up period (1 year).

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Patients A and B and HCMV, based on competitive PCR and fluorometric detection, was developed and applied to 317 samples from a cohort of HIV-infected patients (n = 37) and a cohort of LTRs (n = 26). Our data indicated a generally low reactivation rate of HCMV in the PBL of these immunosuppressed patients. HHV-6 was not detected in any of the 26 LTRs during the first 6 months after transplantation, despite a high reactivation rate of HHV-6 in the PBL of these immunosuppressed patients. HHV-6 DNA detected at the same time point during the follow-up period (1 year).

The high rate of HCMV DNA detection and histopathologically proven HCMV pneumonitis, despite early ganciclovir prophylaxis, in our cohort of LTRs is in keeping with the highly immunosuppressed nature of these patients and makes the lack of HHV-6 DNA detection even more striking. In contrast, our cohort of HIV-infected patients was recruited at a time when HAART was becoming commonplace, and, hence, it is not surprising that the rates of HCMV detection and HCMV disease were so low in this cohort. Nevertheless, the only 2 HHV-6 DNA–positive samples came from the HIV cohort and did not occur in patients who developed HCMV reactivation or disease either at the time that the HHV-6 was detected in the PBL or during the entire follow-up period.

Overall, the prevalence of HHV-6 in the PBL of our immunosuppressed patient groups was relatively low, and our results suggest that HHV-6 is not likely to be an important pathogen in LTRs who are at risk for HCMV disease while receiving ganciclovir prophylaxis. Much of the published literature has concentrated on organ transplant recipients who are at risk but who are not a primary mismatch for HCMV, to avoid the confounding effects of ganciclovir prophylaxis [17–21]. This was not the case for our cohort of LTRs. Hence, our results suggest that there may be a significant difference in viral replication kinetics between the 2 viruses, such that the use of ganciclovir prophylaxis and treatment may be differentially effective against HCMV and HHV-6. A significant number of samples (n = 51) was taken within 6 weeks of lung transplantation, which is both the time of maximum immunosuppression and ganciclovir prophylaxis. HHV-6, like HCMV, is sensitive to ganciclovir and foscarnet and less so to acyclovir [30–32]. In the HIV cohort, we found that HHV-6 may be a possible culprit in HCMV-like disease episodes.

It is highly unlikely that the low detection of HHV-6 in our patient cohort is a result of an insensitive assay but rather is a true indication of HHV-6 reactivation in the PBL of these immunosuppressed patients. The use of an internal control molecule in our assay allowed high sensitivity to be achieved (detection threshold, 10 HHV-6 DNA copies/μg). The PCR assay produced amplified PCR fragments of the appropriate size when tested against common strain HHV-6 variants A1102 and B220, was predicted to be highly specific for HHV-6, and failed to cross-react with other herpesviruses.

Although our findings are somewhat paradoxical in light of recent publications that have increasingly recognized HHV-6 as a potential opportunistic pathogen and have shown a high degree of association between HHV-6 reactivation and HCMV reactivation in immunosuppressed patients [24, 26, 33, 34], there may be several potential explanations for this discrepancy. As mentioned, the confounding effects of ganciclovir prophylaxis in an LTR cohort or HAART-associated immune reconstitution in our HIV cohort are likely to be very important. Second, it is possible that, for any degree of net immunosuppression, the specific immune control mechanisms for different herpesviruses may vary considerably between different cohorts of immunosuppressed patients. This would greatly influence the likelihood of viral reactivation. In addition, HHV-6 and HCMV DNA levels were measured in the PBL and may only partially reflect what is happening in the compartments where either HCMV or HHV-6 disease can potentially occur—the allograft, gastro-

### Table 4. Human cytomegalovirus (HCMV) and human herpesvirus (HHV)–6 DNA detection in peripheral blood leukocytes (PBL) of lung transplant recipients (LTRs).

<table>
<thead>
<tr>
<th>HHV-6 detected</th>
<th>HCMV detected</th>
<th>HCMV not detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n = 26)</td>
<td>Yes 0 0</td>
<td>16b 10</td>
</tr>
<tr>
<td>PBL samples (n = 145)</td>
<td>Yes 0 0</td>
<td>101</td>
</tr>
</tbody>
</table>

* No. of LTRs with HCMV and/or HHV-6 DNA detected at any time point in the follow-up period (6 months).

### Table 5. Human cytomegalovirus (HCMV) and human herpesvirus (HHV)–6 DNA detection in peripheral blood leukocytes (PBL) of human immunodeficiency virus–infected patients.

<table>
<thead>
<tr>
<th>HHV-6 detected</th>
<th>HCMV detected</th>
<th>HCMV not detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n = 37)</td>
<td>Yes 0 2b</td>
<td>10 27</td>
</tr>
<tr>
<td>PBL samples (n = 172)</td>
<td>Yes 0 7</td>
<td>29 136</td>
</tr>
</tbody>
</table>

* No. of patients with HCMV and/or HHV-6 DNA detected at any time point in the follow-up period (1 year).

b See text for clinical details.

c Four patients developed HCMV disease.

d No. of samples with HCMV and/or HHV-6 DNA detected at the same time point during the follow-up period.
infectious disease. The ubiquitous nature of HHV-6 and HCMV in various tissues [35, 36] makes simultaneous detection of both important given the differential tropism of these viruses for the intestinal tract, and central nervous system. This is particularly important as reactivating HHV-6 and HCMV in our 2 cohorts of immunosuppressed patients may be explained, in part, by a significant difference in the background prevalence of these common DNA viruses, although this would seem to be unlikely given their ubiquitous nature.

The simultaneous detection of both HHV-6 and HCMV in urine or serum in renal transplant recipients who were not receiving ganciclovir prophylaxis is a strong predictor of HCMV disease and disease severity [18–19]. Although the use of OKT3 immunosuppression is likely to be an important factor in this association, the relationship between reactivating β-herpesviruses and the transactivating potential of HHV-6 also must be considered. In vitro studies have shown transactivation among related herpesviruses [23–25], which suggests that HHV-6 transactivation of HCMV is a clinical possibility. However, our results suggest that it is not necessarily the case in our 2 clinical cohorts, although clearly we cannot exclude the possibility of sequential β-herpesvirus reactivation. If sequential reactivation is a possibility, then one may expect that a pooled test sample such as urine, rather than a one-time blood sample, may be more sensitive for virus detection.

We conclude that HHV-6 reactivation likely results from a complex interplay of HHV-6 exposure history and prior immunity, degree and type of immunosuppression, DNA viral replication kinetics, and the use of antiviral prophylaxis plus individual case scenarios for the development of allograft rejection syndromes and HIV disease status in organ transplant recipients and HIV-infected patients. Prospective studies are needed to examine cohorts of patients with varying degrees of immunosuppression, at multiple time points, and in several key components to further elucidate the pathobiology, transactivation potential, clinical impact, and potential for therapeutic intervention of HHV-6 reactivation/reinfection.

Acknowledgments

We thank the Victorian Infectious Disease Reference Laboratory and the Prince of Wales Hospital (New South Wales), for providing human herpesvirus (HHV)-6–positive controls, and Theo Sloots (Sir Albert Sakzewski Virus Research Centre, Royal Children’s Hospital, Queensland), for HHV-6 variant typing.

References