Betaherpesviruses in transplant recipients

P. D. Griffiths*, D. A. Clark and V. C. Emery

Department of Virology, Royal Free and University College Medical School (Royal Free Campus), Pond Street, London NW3 2QG, UK

The three betaherpesviruses known to infect humans are cytomegalovirus (CMV) and human herpesviruses 6 and 7 (HHV-6 and -7). All three viruses can infect opportunistically after organ transplantation. CMV causes a variety of end-organ diseases, including pneumonitis, hepatitis and gastrointestinal ulceration. Patients who develop overt CMV disease have significantly higher CMV viral loads than infected patients without evidence of clinical disease. A high CMV viral load largely explains the previously described risk factors for the development of CMV disease, which include donor/recipient serostatus before transplant and viraemia after transplant. CMV also causes some cases of allograft rejection, which can be prevented by antiviral prophylaxis. Application of similar quantitative methods for the study of HHV-6 and -7 have shown that HHV-6 and CMV are significantly and independently associated with biopsy-proven graft rejection after liver transplantation. The full clinicopathological significance of the betaherpesviruses may, thus, be greater than is currently appreciated.

Introduction

The sub-family of betaherpesviruses includes cytomegalovirus (CMV) and the closely related human herpesviruses 6 and 7 (HHV-6 and -7). This paper will summarize new insights into the natural history and pathogenesis of CMV disease provided by recent quantitative studies of CMV viral load. It will also review all prospective studies of HHV-6 and -7 infection after solid organ and bone marrow transplantation.

Cytomegalovirus

CMV infects approximately 60% of adults in developed countries and almost all of those in developing countries. The virus can be acquired by: (i) intrauterine infection (approximately 1%); (ii) perinatal transmission from the mother to child, either through exposure to maternal genital secretions or to breast milk (5–20%); or (iii) by close contact between individuals, including the transfer of saliva and other body fluids during sexual intercourse. In addition to these natural routes of transmission, iatrogenic transmission can occur during blood transfusion, from donated solid organs or from donor semen samples. Any individual with CMV IgG antibodies is at risk of reactivating latent virus. Seropositive individuals (who are infected with one strain of CMV) can be reinfected with a second strain of CMV; this typically occurs when a seropositive donor organ is transplanted into a seropositive recipient.

CMV causes a variety of diseases in the immunocompromised transplant patient (Table I), although the anatomical sites most commonly affected vary between patient groups. For example, pneumonitis is a major problem after bone marrow transplantation but is less common in solid organ recipients. This may result from CMV triggering abnormal immune responses within the lung such that disease is caused indirectly by the virus.1 Likewise, a myelosuppressive syndrome occurs in bone marrow transplant recipients, where the pathogenesis is thought to involve interference with release of growth factors that are required for normal haematopoiesis.2 In addition to these direct end-organ diseases, CMV has repeatedly been associated with rejection of solid organs, or graft-versus-host disease (GVHD) in the case of bone marrow transplantation. The phenomenon of graft rejection, atherosclerosis after heart transplants, chronic obliterative bronchiolitis after lung transplant and secondary bacterial and fungal infections are all frequent after CMV disease and are termed ‘indirect effects’ of the virus.3

Risk factors for CMV disease

To study cases of CMV end-organ disease (termed ‘CMV disease’ throughout this article), an internationally agreed
a marked transition once the peak viral load exceeded $5 \log_{10}$ genomes/mL of urine; a phenomenon we have termed the ‘threshold concept of CMV disease’. Subsequent analyses using CMV load in the blood of 97 renal transplant recipients confirmed these findings, that is, that peak CMV load is the predominant risk factor for CMV disease.\textsuperscript{6}

We conducted a similar study in 162 liver transplant recipients with a total of 1433 surveillance blood samples.\textsuperscript{7} Of 51 patients in whose blood CMV DNA was detected, 20 developed CMV disease. Of the patients with no detectable CMV DNA in their blood, none developed CMV disease ($P < 0.0001$). The peak viral load across all patients during the period after the transplant ranged from 3.59 to 7.57 $\log_{10}$ genomes/mL blood, but was significantly higher in patients with CMV disease. Univariate analysis showed that a high viral load and donor seronegativity were risk factors for CMV disease with odds ratios of 2.22 (95% CI 1.37–3.59) for each 0.25 $\log_{10}$ increase in viral load and 4.11 (95% CI 1.02–16.67), respectively. However, in bivariate logistic regression models, only a high viral load remained a significant risk factor for CMV disease. Interestingly, the additional factor of receipt of augmented methylprednisolone for treatment of graft rejection was shown to be a risk factor independent of CMV load (odds ratio 1.61 (95% CI 1.04–2.51); $P < 0.03$). It was possible to illustrate this relationship mathematically by showing that the sigmoid curve representing the threshold effect of CMV disease was shifted to the left by approximately 0.4 $\log_{10}$ for each 3 g course of methylprednisolone administered.\textsuperscript{7} This suggests that administration of methylprednisolone makes patients more susceptible to disease at a given peak CMV load. Thus, a 50% probability of disease is reached at 5.6 $\log_{10}$ genomes/mL of blood in the absence of augmented methylprednisolone but shifts to 4.5 $\log_{10}$ genomes/mL when a cumulative dose of 9 g of methylprednisolone is administered.

Since risk factors for CMV disease differ between solid organ and bone marrow transplant recipients, we performed similar analyses in bone marrow transplant patients.\textsuperscript{8} Prospective follow-up of 110 bone marrow recipients with a total of 1647 surveillance blood samples identified 49 patients with CMV DNA in their blood, of whom 15 experienced CMV disease. Consistent with the data obtained in the solid organ transplant groups described above, peak CMV load was greater in symptomatic patients (median 4.5 $\log_{10}$ genomes/mL) than in asymptomatic patients (median 3.6 $\log_{10}$ genomes/mL; $P < 0.002$). Peak CMV load was also significantly greater in CMV-seropositive recipients of seronegative marrow (D’R\textsuperscript{+}; median 5.0 $\log_{10}$ genomes/mL) than in patients in the D’R\textsuperscript{+} and D’R\textsuperscript{−} groups ($P < 0.01$ and $P < 0.005$, respectively). It should be noted that the bone marrow transplant patients at greatest risk of CMV disease are recipient seropositive (because they reactivate their own virus) who receive marrow from seronegative donors (cf. solid organ recipients). This may be a consequence of adoptive transfer of some protective immunity.
Betaherpesviruses in transplant recipients from an immune donor at the time of marrow harvest. In univariate analysis, increased CMV load was the most significant risk factor for CMV disease (odds ratio 1.43 (95% CI 1.12–1.82); \( P = 0.004 \) for each 0.25 log<sub>10</sub> increase in viral load), although both D–R patients and those suffering acute GVHD were also at increased risk of CMV disease (odds ratios 6.60 (95% CI 0.95–15.72), \( P = 0.05 \); and 3.17 (95% CI 0.87–11.55), \( P = 0.08 \)). However, as for data presented previously, only increased CMV load remained significant in multivariate analysis (\( P = 0.006 \)).

Results from all of these patient groups imply that viral replication at a local site can overwhelm local immune responses and lead to viraemic dissemination of CMV to multiple target organs. Viraemia itself does not necessarily guarantee the development of CMV disease because the local immune response within each organ may be able to control viral pathogenesis at each site. However, if the viral load increases, it may overcome host defences and precipitate disease. By studying serial measures of viraemia in transplant patients, it has been possible to calculate the rate of CMV replication and clearance in the immunocompromised host. Studies have demonstrated that CMV replicates dynamically with a doubling time in vivo of approximately 1 day during an active infection. This indicates that the reputation of CMV as a slowly growing virus is incorrect. Certainly, the time taken to produce a cytopathic effect in cell cultures is long, but this should not be taken as evidence of slow replication in vitro or in vivo. This conclusion has important practical applications with respect to the potency of antiviral drugs necessary to control CMV replication and CMV disease.

**HHV-6 and HHV-7**

HHV-6 was first isolated in 1986 and has been shown to be a cause of exanthem subitum and febrile fits. HHV-7 was isolated in 1990 and is also a cause of exanthem subitum. DNA sequencing has shown that these two members of the Betaherpesvirinae are closely related genetically. Although also related closely to human CMV, both HHV-6 and -7 lack the genes encoded in the unique short region of CMV. The relevance of this genetic difference to the pathogenesis of HHV-6 and -7 is unknown, but many of the genes involved in down-regulating class I HLA genes during CMV infection are within the unique short region.

Individual case reports of diseases associated with HHV-6 after transplants have appeared in the literature, with the most convincing being encephalitis and, possibly, bone marrow suppression. Several cohort studies have described the incidence of HHV-6 (and, sometimes, HHV-7) infection after solid organ or bone marrow transplantation (Tables II and III) and these illustrate that both viruses occur frequently after transplantation. However, the analysis of disease associations has generally involved the study of individual cases of disease occurring simultaneously.

### Table II. Detection of HHV-6 and -7 after solid organ transplantation

<table>
<thead>
<tr>
<th>Reference</th>
<th>Method</th>
<th>Virus</th>
<th>Organ transplant</th>
<th>Antiviral prophylaxis</th>
<th>Number of serial bloods</th>
<th>Observed clinical disease</th>
<th>Case reports</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schmidt et al.</td>
<td>PCR</td>
<td>HHV-6</td>
<td>liver</td>
<td>immunoglobulin</td>
<td>46</td>
<td>CMVD</td>
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<td>Herbein et al.</td>
<td>virus isolation</td>
<td>HHV-6</td>
<td>liver</td>
<td>NG</td>
<td>11</td>
<td>none</td>
<td>none</td>
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<td>Osman et al.</td>
<td>PCR</td>
<td>HHV-6</td>
<td>kidney</td>
<td>NG</td>
<td>18</td>
<td>NG</td>
<td>NG</td>
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<td>Griffiths et al.</td>
<td>PCR</td>
<td>HHV-6</td>
<td>multiple organ</td>
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<td>Griffiths et al.</td>
<td>PCR + QCPCR</td>
<td>HHV-6</td>
<td>kidney</td>
<td>NG</td>
<td>56</td>
<td>NG</td>
<td>536</td>
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</tbody>
</table>

**Abbreviations:** CMVD, increased CMV disease; NG, information not given in the cited publication; QCPCR, quantitative competitive PCR.

Effects on whole population.
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Table III. Detection of HHV-6 and -7 after bone marrow transplantation

<table>
<thead>
<tr>
<th>Reference</th>
<th>Virus</th>
<th>Method</th>
<th>Number of patients</th>
<th>Number of blood samples</th>
<th>Antiviral prophylaxis</th>
<th>Observed diseases a</th>
<th>Case reports</th>
</tr>
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<tr>
<td>Wilborn et al. 23</td>
<td>HHV-6</td>
<td>PCR</td>
<td>57</td>
<td>415</td>
<td>NG</td>
<td>none</td>
<td>GVHD</td>
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<td>Kadakia et al. 26</td>
<td>HHV-6</td>
<td>Virus isolation</td>
<td>26</td>
<td></td>
<td>NG</td>
<td>no association with engraftment</td>
<td></td>
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<tr>
<td>Wang et al. 25</td>
<td>HHV-6</td>
<td>PCR</td>
<td>37</td>
<td>270</td>
<td>high-dose ACV (n = 9) b</td>
<td>none</td>
<td>engraftment</td>
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<tr>
<td>Appleton et al. 24</td>
<td>HHV-6</td>
<td>PCR + IHC</td>
<td>57</td>
<td></td>
<td>moderate dose ACV</td>
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<td>GVHD c</td>
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<tr>
<td>Chan et al. 27</td>
<td>HHV-6 and -7</td>
<td>PCR</td>
<td>61</td>
<td>563</td>
<td>NG</td>
<td>none</td>
<td>engraftment</td>
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<tr>
<td>Cone et al. 28</td>
<td>HHV-6</td>
<td>PCR</td>
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<td>264</td>
<td>low-dose ACV (n = 15)</td>
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<td>rash</td>
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</table>

Abbreviations: ACV, acyclovir; GVHD, graft versus host disease; IHC, immunohistochemistry; NG, information not given in the publication cited.

aEffects on whole population.
bNumbers in parentheses indicate how many patients were given the specified prophylaxis.
cSkin, not blood.

case associations that might have occurred by coincidence for example, some studies report GVHD to be associated with HHV-6 whereas others do not. Over-all, these cohort studies report a low incidence of disease previously identified by case reports of HHV-6, such as encephalitis, bone marrow suppression or pneumonitis. However, it may be premature to conclude that these viruses are not pathogens after transplantation.

A confounding factor in the analysis of HHV-6 and -7 present in blood and other organs using PCR-based methods is the potential for detection of latent virus. For example, using 1 ng of DNA extracted from blood and a highly sensitive, nested PCR approach, HHV-6 and -7 can be detected in 45% and 83% of the population, respectively.29 To avoid detecting latent virus, we used a quantitative approach and a relatively low input quantity of DNA (equivalent to approximately 6000 cells) for all our analyses. QCPCR assays for HHV-6 and HHV-7 were also used to define whether high viral loads are associated with clinical parameters such as duration of fever, duration of abnormal liver function tests, as well as indirect effects such as graft rejection.

A series of 60 liver transplant patients were recruited as a result of the engineering of a unique restriction site in the target sequence of HHV-6 or -7. Chimpanoepidemiological analyses have shown that all three viruses are significantly associated with biopsy-proven graft rejection.22 Taken together, these results suggest that HHV-6 may be a previously unrecognized pathogen in liver transplant patients, with biopsy-proven graft rejection being the major clinicopathological outcome. Thus, HHV-6, like CMV, may indirectly trigger harmful sequelae; this should be a previously unrecognized pathogen in liver transplant patients. Given the complexities of these medical cases, it is to be expected that some of the reported disease associations might have occurred by coincidence; for example, some studies report GVHD to be associated with HHV-6 whereas others do not. Over-all, these cohort studies report a low incidence of disease previously identified by case reports of HHV-6, such as encephalitis, bone marrow suppression or pneumonitis. However, it may be premature to conclude that these viruses are not pathogens after transplantation.

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taken into account when studying all betaherpesviruses. In addition, placebo-controlled trials of anti-betaherpesvirus compounds should investigate whether clinical benefits observed result from inhibition of CMV, HHV-6 or HHV-7. For example, a recent report showed a 50% reduction in acute graft rejection after 90 days of prophylaxis with high-dose valaciclovir. The authors concluded that this reduction may have resulted from inhibition of CMV. While we agree that this is a likely proposition, the possibility that HHV-6 and -7 may also contribute to graft rejection under these circumstances should not be excluded.

Given that HHV-6 and -7 have many genes in common with CMV, we tried to determine whether the product of the U69 gene of HHV-6, a homologue of the CMV UL97 gene, could specifically phosphorylate ganciclovir and act as a classical protein kinase. Expression of U69, using recombinant baculoviruses followed by enzymic and biochemical characterization, showed that the U69 gene could confer ganciclovir susceptibility to baculovirus replication (an organism not usually susceptible since ganciclovir is not effectively phosphorylated to the monophosphate in insect cells). In addition, the product of the U69 gene autophosphorylated on serine residues and could catalyse the transfer of phosphates on to exogenous serine/threonine protein kinase substrates. Overall, these data show that the U69 gene can act in a similar way to the CMV UL97 gene in the activation of ganciclovir and are consistent with the in vitro and limited in vivo data indicating that HHV-6 replication can be inhibited by ganciclovir in a dose-dependent manner. The development of the benzimidazole class of compounds as inhibitors of the CMV UL97 protein kinase function may allow the development of similar compounds that inhibit the U69 kinase function and, hence, HHV-6 replication.

Conclusions and future prospects

The pathogenicity of the betaherpesviruses can be ranked in the order CMV > HHV-6 > HHV-7, and accumulating evidence suggests that HHV-6 and -7 are associated with graft rejection after transplantation. It is important to determine whether these viruses are the trigger for such rejection or whether they are merely activated as a consequence of the immunological events surrounding rejection. Placebo-controlled trials with compounds that are known to inhibit HHV-6 and -7 in vivo are clearly required to differentiate these possibilities. The results of a recent placebo-controlled trial of valaciclovir in renal transplant recipients has confirmed the benefit of this agent in reducing CMV disease and in significantly reducing rejection episodes. Our increasing understanding of the dynamics of replication of these viruses in the human host should enable the further development of rational and potentially novel therapies with the ability to improve patient management.

References


