Cytomegalovirus, Human Herpesvirus-6, and Human Herpesvirus-7 in Hematological Patients

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The prototype member of the Betaherpesvirinae subfamily, cytomegalovirus (CMV), is the most important infectious pathogen in transplant recipients, including those receiving bone marrow or stem cell grafts. Overt CMV disease such as pneumonitis is notoriously difficult to treat. Antiviral prophylaxis, rapid diagnostic tests to identify CMV infection, and preemptive antiviral chemotherapy are significant improvements in the management of CMV. As the kinetics of the immune response to CMV become better defined, immunotherapeutic approaches should be introduced to complement current management strategies. Two newly identified betaherpesviruses, human herpesvirus-6 (HHV-6) and human herpesvirus-7 (HHV-7), are genetically more closely related to each other than to CMV. Both are highly prevalent in the general population and infections post–bone marrow transplantation are common. These viruses are not as pathogenic as CMV but HHV-6 at least can cause disease such as encephalitis, hepatitis, and bone marrow suppression. Both of these newer herpesviruses are potentially susceptible to existing and licensed antiherpesvirus drugs.

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Cytomegalovirus

CMV infects approximately 60% of adults in developed countries and almost all of those in developing nations. The virus is usually acquired by close contact between individuals, mainly by the transfer of saliva but also through intrauterine or perinatal infection. In addition, iatrogenic transmission can occur, as from donated solid organs and through blood transfusion.78 Primary infections are usually asymptomatic although delayed infection in adulthood can cause infectious mononucleosis–like illness.

CMV establishes life-long infection after primary infection. Persistence likely includes both a latent state with infectious virus only produced during episodes of reactivation, and chronic replication with continuous or frequent but intermittent production of infectious virus. Monocyte/macrophages and endothelial cells are implicated as sites of CMV persistence and latency, and replication of the virus in these two cell types is likely to be important in maintaining life-long infection.42,82,87

CMV causes a variety of diseases in the immunocompromised host (Table 1), although the anatomic sites most commonly affected vary among patient groups. 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 bone marrow transplantation. Secondary bacterial and fungal infections also are frequent after CMV infection and, together with rejection and GvHD, are considered indirect effects of the virus.79

CMV Infection Following Bone Marrow Transplantation

Historically, CMV has been one of the most feared infectious complications following bone marrow transplantation. CMV disease in this setting can manifest as pneumonitis, gastrointestinal syndromes, hepatitis, bone marrow suppression, and occasionally retinitis (Table 1). The lungs of the recipients become a site for CMV replication, and CMV pneumonitis was one of the major diseases following transplantation which, once established, led to high morbidity and mortality. Prior to the advent of more rapid diagnostic methods, the slow growth of CMV in vitro allowed a diagnosis of active CMV infection only late in the clinical course. Under these conditions, antiviral therapeutic intervention was of limited success.74 As discussed below, in more recent years antiviral prophylaxis36 and/or improved diagnostic methods and correct deployment of antiviral chemotherapy against CMV22 have dramatically re-
duced the incidence of CMV pneumonitis in hematol-ogy patients and also led to improved outcomes in infected patients.

CMV Replication and Viral Burden
CMV has the reputation of a slowly growing virus. While true in conventional in vitro culture systems in primary human fibroblasts, it is unlikely that such an artificial system parallels in vivo complexity. The recent application of dynamic mathematical modeling has illuminated our understanding of the replication rate of many human pathogens in vivo. Using such analyses, we have shown that CMV replication in vivo occurs rapidly, with doubling times of approximately 1 day.23 These findings have implications for the development of disease and the successful treatment of infection in patients with hematological malignancies undergoing bone marrow transplantation.

Many risk factors for CMV disease have been defined in prospective studies spanning 20 years. Using conventional cell culture methods and subsequently rapid culture methods such as shell vial culture and the detection of early antigen fluorescent foci (DEAFF) test, active CMV replication in blood (viremia) was associated with an increased risk of development of CMV disease post–bone marrow transplantation.46,64,93 Subsequently, the detection of virus using more sensitive methods such as antigenemia assays (where polymorphonuclear cells expressing the ppUL83 protein of CMV are identified using specific monoclonal antibodies) and the polymerase chain reaction (PCR) have shown that both CMV presence and the quantity of virus found in the blood are risk factors for CMV disease.4,7,21,25,31,52,73,86

The traditional major predictor for a high risk of CMV disease has been the transplantation of donor marrow from CMV-seronegative individuals into a recipient who is already CMV-seropositive. Under such conditions, the donor marrow is naive to CMV, and when endogenous CMV is reactivated the marrow-derived immune cells mount a primary immune response to control replication. In contrast, donor and recipient seropositivity for CMV is associated with a lower risk of CMV disease, probably due to the transfer of antiviral immunity with donor marrow.35 These qualitative effects of donor-recipient serostatus manifest themselves quantitatively through viral load, such that patients in the D−R− category exhibit higher viral loads (and hence a higher probability of disease) post-transplantation compared to patients who are in the D+R+ or D−R+ categories (Fig 1).31 Regression of the probability of CMV disease with viral load showed an acute transition at critical quantities of virus, resulting in a large increase in disease risk for relatively small increases in viral load. As a consequence, viral load levels can be defined that identify patients at risk of disease with reasonable sensitivity and specificity. The advent of real-time PCR monitoring of viral load is allowing these concepts to be translated practically, to identify patients at risk of future CMV disease and to initiate therapy in a timely, cost-effective manner.60,67,73,86

Immune Control of CMV Following Bone Marrow Transplantation
T cells are critical in the control of CMV replication. Extensive research has shown that the cytotoxic T lymphocyte (CTL) response to CMV is dominated by activity against the pp65 (ppUL83) tegument protein, with contributions from T cells directed against the immediate early 1 protein among others.95 In the immunocompetent host, CD8 T cells specific for ppUL83 can be visualized using human leukocyte...
antigen (HLA) tetramer technology (~1%), and in bone marrow transplant recipients undergoing high-level CMV replication, frequencies as high as 20% of the total CD8+ T-cell population have been observed. Using a series of phenotypic markers, these CD8 T cells have a late effector phenotype (CD45RO+, CD27−, CCR7−) and are therefore fully differentiated cytotoxic T cells. A large proportion of CMV-specific CD8 T cells reside in the CD45RA population traditionally regarded as naive T cells. For CD4 T-cell helper responses, there is a predominance in responses against glycoprotein B (gB, UL55; a major target for neutralizing antibody response) and ppUL83, although individual epitopes restricted by HLA class II alleles have not been as extensively mapped as have HLA class I–restricted peptides. Nevertheless, in immunocompetent individuals the T helper responses against total CMV antigens also are relatively high and appear to be important to maintain CMV-specific CD8 T cells.

In the context of bone marrow transplant, the engrafting marrow must cope with a range of infectious agents experienced by the stem cell recipient. CD4 T-helper cells regenerate relatively slowly following allogeneic bone marrow transplantation, and so appropriate help to CD8 T cells able to control CMV replication may be limited. The importance of the regeneration of T-cell immunity after bone marrow transplantation for the control of CMV replication has been demonstrated in studies of CMV-specific T cells and by adoptive transfer of CMV-specific T cells. HLA tetramer technology has improved our understanding of the kinetics of regeneration of CD8-specific CTL responses against CMV following transplantation and provided insight into the level of CD8 T cells required to ensure that CMV infection is maintained at very low levels. An absolute CMV CD8 T-cell level of approximately 10^8 cells/L was associated with total suppression of CMV replication, at least at the level of sensitivity of the PCR assay employed (~200 genomes/mL blood). However, in patients who experienced a period of active CMV infection (PCR positivity > 200 genomes/mL blood), the median CMV CD8 T-cell frequency was substantially lower, at approximately 10^7 cells/L, whereas during periods where replication was suppressed, the median frequency increased to about 3 × 10^7 cells/L. From these and other data it would appear that certain threshold levels of CMV CD8 T cells may be required to effectively suppress CMV replication; when the absolute number of cells is below this threshold, high levels of CMV replication ensue, leading to a high risk of disease.

A variety of mechanisms might render the CMV CD8 T-cell population suboptimal for control of viral replication, including slow reconstitution of T-helper responses in the post-transplant period and the effects of immunosuppressive drugs such as corticosteroids used to control GvHD. Prolonged exposure to prednisolone is associated with relentless suppression of CMV specific CD8 T cells and a consequent increase in viral load (Fig 2). The powerful effect of CMV-specific T cells in limiting CMV replication was dramatically demonstrated when an adoptive immunotherapeutic approach achieved suppression of CMV replication in patients who had high viral loads that were unresponsive to conventional antiviral chemotherapy. Studies are in progress to determine whether a combination of viral load measurement and CMV-specific CD8 T-cell determinations might provide complementary information to identify patients at high risk of developing CMV infection and disease following transplantation.

Therapeutic Interventions Against CMV Following Bone Marrow Transplantation

Two anti-CMV agents have been the mainstay for the treatment of established disease in the hematology patient: ganciclovir (an acyclic guanosine analogue) and foscarnet (an analogue of inorganic pyrophosphate). Both block activity of CMV DNA polymerase (UL54 gene), ganciclovir by acting as a competitive inhibitor of the natural substrate and thus causing chain termination, and foscarnet as a product inhibitor of the DNA synthetic reaction catalyzed by the
viral-specific DNA polymerase. For ganciclovir to be effective it must be phosphorylated to the triphosphate moiety; the first stage of phosphorylation is effected by a viral specific protein kinase encoded by the UL97 gene, while subsequent phosphorylation steps are catalyzed by cellular kinases. Ganciclovir therapy (5 mg/kg twice daily) has an antiviral efficacy of approximately 91.5% at controlling CMV replication.23 Similar estimates for the efficacy of foscarnet are not available, but trials comparing ganciclovir with foscarnet indicate much similarity.76 The effectiveness of preemptive therapy triggered by antigenemia positivity or PCR positivity has been shown in two trials,6,10, in both the treatment was initiated far earlier than using conventional cell culture methods and patients required less overall ganciclovir therapy (5 mg/kg twice daily), have produced clinical benefit6,10; in both the treatment was initiated far earlier than using conventional cell culture methods and patients required less overall ganciclovir therapy (5 mg/kg twice daily), have produced clinical benefit.6,10 Therapy up to 100 days following bone marrow engraftment is effective at controlling CMV infection and its consequent disease. However, there are a variety of ramifications to these strategies. First, since CMV replication is substantially inhibited by oral ganciclovir (this formulation has an antiviral efficacy of approximately 46.5%),53 the regenerating immune system is not exposed to sufficient levels of viral replication to elicit a T-cell–mediated immune response, and so patients remain unprimed to CMV following the cessation of prophylaxis.60 Second, because the drug concentration is inadequate to fully eradicate replication during the period of prophylaxis, coupled with the lack of regeneration of an early immune response against CMV, viremia can appear following the cessation of prophylaxis (usually between 3 and 4 weeks) especially in the high-risk D “R” group. Unfortunately, late CMV infection has been associated with a high incidence of late CMV disease,88 which has been difficult to manage, stimulating many centers to pursue aggressive preemptive therapy triggered by antigenemia or PCR positivity.

Although drug resistance is an emerging question in the context of CMV,24,51 a recent report has shown that antigenemia-driven preemptive therapy with ganciclovir did not produce ganciclovir-resistant viruses: there was no selection of viruses with mutations in UL97, despite recurrence of antigenemia.28 A major risk factor for the recurrent viremia appears to be the slope of decline in viral load during initial therapy, so that a tardy viral load response to intervention increases the likelihood of subsequent viremic episodes.38

Intravenous and oral formulations of ganciclovir have been used extensively, but the prodrug of ganciclovir, valganciclovir is now available.62,71 Although there are no results from controlled trials of this compound in bone marrow transplant recipients, the prodrug should provide levels of ganciclovir in the blood that are comparable to intravenous ganciclovir, but attention to poor absorption may be necessary in the presence of gut GVHD. Valganciclovir likely will replace intravenous ganciclovir. Since the effective concentration of ganciclovir has been increased over oral ganciclovir, the antiviral efficacy achieved should be also be greater. How use of valganciclovir in treatment and prophylaxis will affect late infection and generation of ganciclovir-resistant viruses remains to be defined, but analysis of samples obtained from a clinical trial of valganciclovir versus intravenous ganciclovir in HIV-infected individuals showed that the frequency of ganciclovir-resistant strains of CMV were comparable between the two treatment groups.8

Human Herpevirus-6

HHV-6 is highly prevalent worldwide and infection usually occurs in early childhood. In young children, HHV-6 is a causative agent of febrile illness including exanthem subitum (ES).97 Two variants of HHV-6 have been defined (termed A and B), and they are increasingly viewed as closely related although distinct species.18 HHV-6 is tropic for T lymphocytes and neural cells but has the ability to infect a wide variety of cell types in vitro.13 HHV-6 utilizes CD46 as a cellular receptor (CD46, also termed membrane cofactor protein, is a member of a family of complement regulators).80 Activated CD4+ T cells and monocytes/macrophages appear to be the preferential targets for replication in vivo.98,83 Similar to CMV, HHV-6 remains in the host lifelong following primary infection. The virus, predominantly variant B, can be detected in the peripheral blood of healthy individuals by nested PCR when sufficient quantities of DNA are tested.15 The actual site of latency has yet to be established, although candidates include monocytes37 and early bone marrow progenitor cells.8 An uncommon form of
HHV-6 persistence is characterized by integration of apparently whole-genome length HHV-6 sequences into host-cell chromosomes, but the clinical relevance of this unusual biology, if any, is not clear. Integration sites have been mapped to chromosome 17p13.3, chromosome 22q13, and chromosome 1q44 in different individuals. The inheritance of chromosomally integrated HHV-6 also has been reported.

HHV-6 and Lymphoproliferative Disease

The involvement of HHV-6 in a range of hematological malignancies has been investigated with largely negative findings. In situ detection of HHV-6 has been demonstrated in the abnormal cells of T-cell chronic lymphoproliferative disease and sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease). Further studies are necessary to determine if chromosomally integrated HHV-6 also has been reported.

HHV-6 Infection Following Bone Marrow Transplantation

Infection with HHV-6 following bone marrow transplantation is common and, considering the high rate of seroprevalence of the population, is likely to be due almost always to reactivation of recipient’s virus or reinfecion from the donor. The virus has been found in 28% of bone marrow samples from healthy individuals, suggesting that it can be transmitted from the donor to the recipient, and cases of primary infecon have been reported. HHV-6A and especially HHV-6B infections have been detected in the post-transplant period; most HHV-6 infections occur within the first 4 weeks.

Case reports and selective studies have associated HHV-6 with a range of clinical diseases following bone marrow transplantation, including encephalitis, idiopathic interstitial pneumonitis, hepatitis, early and late graft failure, and bone marrow suppression. In vitro, both HHV-6 variants are able to suppress the maturation and growth of normal bone marrow precursors, including granulocyte/macrophage, erythroid, and megakaryocytic lineages. The detection of HHV-6 in cerebrospinal fluid by PCR also has been associated with a range of CNS disease in bone marrow transplant recipients.

Prospective studies have been conducted to measure the medical impact of HHV-6 following stem cell transplantation (Table 2). Some have reported associations between HHV-6 and delayed engraftment, myelosuppression, and fever. HHV-6 viral load also significantly correlated with delayed platelet engraftment following stem cell transplantation. However, other analyses have failed to show a clear association between HHV-6 and disease when the whole study population was analyzed. Subgroup analysis sometimes has linked the virus to GvHD, delayed engraftment, rash, and rash. Contradictory findings in prospective studies are likely to represent variations in the demographics of transplant populations, their medical care, and, importantly, the methods used in detection of virus infection (although the majority have employed PCR). Most authors have found a low incidence of the disease types previously identified by case reports when large numbers of bone marrow transplant recipients were examined.

Human Herpesvirus-7

As with HHV-6, HHV-7 also is highly prevalent worldwide and infection usually occurs in early childhood, perhaps slightly delayed in comparison to HHV-6. Similar to HHV-6, HHV-7 can cause febrile
illness in young children. Analysis of the HHV-7 genome shows that it is most closely related to HHV-6.

HHV-7 utilizes CD4 as a cellular receptor to infect T cells and although CXCR4 was initially reported to be downregulated in infected cells, more recent studies suggest that this molecule is not a co-receptor. The virus has also been shown to productively infect macrophages in vitro.

Similar to HHV-6, HHV-7 can be detected in the peripheral blood of healthy individuals by nested PCR, although the actual site of latency has yet to be identified. Monocyte/macrophages harbor virus in a nonreplicating form following infection in vitro.

**HHV-7 Following Transplantation**

Fewer studies have examined the role of HHV-7 infection post-transplantation (Table 3). Similar to HHV-6, active HHV-7 infection does occur, likely due to reactivation of recipient’s virus or reinfection, given the high seroprevalence in the population. HHV-7 DNA has been detected by PCR in 50% of bone marrow samples from healthy persons, including CD34+ and CD34- cell fractions, suggesting that donor material may be a potential source of virus.

Prospective studies in bone marrow transplant recipients have not found an obvious correlation between HHV-7 infection and a range of clinical endpoints including GvHD and engraftment (Table 3). The detection of HHV-7 in peripheral blood by PCR during the early post-transplantation period was associated with a longer time to neutrophil engraftment. In vitro HHV-7 was not suppressive of granulocyte/macrophage, erythroid, and megakaryocyte colony formation in some experiments, but inoculation of cord blood CD34+ cells in vitro with HHV-7 significantly decreased the number of pluripotent (granulocyte/monocyte/erythroid/megakaryocyte) progenitors in semisolid assays with a more modest effect on committed (granulocyte/macrophage and erythroid) progenitors. In addition, HHV-7 appeared to hasten differentiation of cord blood CD34+ cells to myeloid but not erythroid cells. Thus, there are in vitro data to suggest the ability of HHV-7 to perturb the maturation of hematopoietic progenitor cells. Two cases of HHV-7 encephalitis, one fatal, following bone marrow or stem cell transplantation have been reported.

A number of prospective studies have followed solid organ transplant patients (Table 3). In renal recipients, concomitant CMV and HHV-7 infection was associated with a greater risk of developing CMV disease (Table 3). Others have reported a similar association between HHV-7 and an increased risk of CMV disease, but putative mechanisms are unknown.

**HHV-6 and HHV-7 Antiviral Susceptibility**

Ganciclovir, foscarin, and cidofovir have been reported to be inhibitors of HHV-6 and HHV-7 replication in vitro, although not consistently. There have been no controlled trials of antiviral therapy against HHV-6 or HHV-7 infection, but individual published cases suggested a clinical response to treatment of HHV-6 disease (encephalitis/CNS disease and bone marrow suppression) after bone marrow transplantation, using either ganciclovir or foscarin or both. Currently licensed antitherpetic compounds may be effective against HHV-6 and HHV-7, but treatment strategies need to be formulated through appropriate clinical protocols.

**Conclusion**

CMV remains an important pathogen in hematological patients, specifically bone marrow/stem cell transplant recipients. However, important advances have been made in the monitoring and treatment of CMV infection in these patients. The future use of immunotherapeutic approaches may complement the current management strategies. The clinical relevance of HHV-6 and HHV-7 in hematological patients is certainly more restricted than CMV, although accumulating evidence highlights the ability of HHV-6 to cause disease post bone marrow/stem cell transplantation.

**References**


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**Table 3. Prospective Studies of HHV-7 Infection Following Transplantation**

<table>
<thead>
<tr>
<th>Study</th>
<th>Transplant Type</th>
<th>Method of Detection</th>
<th>Observed Disease</th>
</tr>
</thead>
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<tr>
<td>Wang et al(1)</td>
<td>Bone marrow</td>
<td>PCR</td>
<td>None</td>
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<tr>
<td>Chan et al(2)</td>
<td>Bone marrow</td>
<td>PCR</td>
<td>Delayed engraftment*</td>
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<tr>
<td>Maeda et al(3)</td>
<td>Bone marrow</td>
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<td>None</td>
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<tr>
<td>Osman et al(4)</td>
<td>Renal</td>
<td>PCR</td>
<td>CMV disease</td>
</tr>
<tr>
<td>Kidd et al(5)</td>
<td>Renal</td>
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<tr>
<td>Tong et al(6)</td>
<td>Renal</td>
<td>PCR</td>
<td>Graft rejection*</td>
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<td>Griffiths et al(7)</td>
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<td>PCR</td>
<td>CMV disease</td>
</tr>
<tr>
<td>Mendez et al(8)</td>
<td>Liver</td>
<td>PCR</td>
<td>None</td>
</tr>
</tbody>
</table>

* Analysis on subgroup of patient population.
37. Hassan-Walker AF, Vargas Cuero AL, Mattes FM, et al: CD8+ cytotoxic lymphocyte responses against cytomegalovirus after liver transplantation: correlation with time from