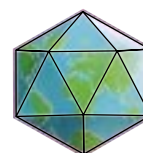


Recommendations from the  
IHMF Management Strategies Workshop  
and 4th Annual Meeting

Editors: Professor PD Griffiths  
Professor RJ Whitley

CYTOMEGALOVIRUS  
AND HUMAN  
HERPESVIRUS TYPE 6  
INFECTIONS IN THE  
IMMUNOCOMPROMISED  
(NON-HIV) HOST



**IHMF**  
International  
Herpes Management  
— Forum —

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The *International Herpes Management Forum* (IHMF) was established to improve the awareness, understanding, counselling and management of infections caused by herpesviruses. Steered by the IHMF Board of Professor Richard Whitley, Dr Martin Wood, Dr Lawrence Corey, Professor Paul Griffiths, Dr Susanne Kroon, Dr Antonio Volpi and Dr Koichi Yamanishi, the IHMF involves international Opinion Leaders in all aspects of medical management of herpesvirus infections including herpes simplex virus (HSV), varicella zoster virus (VZV) and cytomegalovirus (CMV) infections.

The eighth IHMF workshop was held on 29–30 March 1996 to discuss the management of CMV infections in immunocompromised (non-HIV) individuals. This workshop reviewed the pathogenesis and immunobiology of CMV and HHV-6 and evaluated different approaches to the prevention and treatment of CMV including the use of antivirals, developments with vaccines and approaches for the future. Particular attention was focused on the care of the immunocompromised transplant recipient. The aim of the workshop was to improve the management of CMV and HHV-6 and quality of life across all patient populations and to develop recommendations for the best practice of preventing and treating CMV infections and disease.

These draft recommendations were discussed at the 4th Annual Meeting of the IHMF that took place on 9–10 November 1996. This publication, *Cytomegalovirus and Human Herpesvirus Type 6 Infections in the Immunocompromised (Non-HIV) Host*, is part of the series, *Management Strategies in Herpes*. It contains amendments made to the guidelines following extensive discussion at the 4th Annual Meeting.

The editors would like to thank all the participants in the ninth IHMF workshop, and those at the 4th Annual Meeting for their contribution and especially the Co-Chairs of the working groups.

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## The Natural History and Pathogenesis of Cytomegalovirus Infections

Human infection with cytomegalovirus (CMV) is common; between 40% and 100% of the adult population worldwide is seropositive. Many individuals are infected with CMV as children, and in most healthy people infection does not cause significant clinical disease. However, in individuals with an immunodeficiency such as transplant recipients, individuals with HIV infection, or individuals with immature immune systems, CMV infection can lead to severe clinical disease.

### Epidemiology

CMV may be acquired congenitally, perinatally and early postnatally, as well as horizontally through direct physical contact with bodily fluids (saliva, semen, urine), or transplantation of organs and blood transfusions from seropositive donors.<sup>1</sup>

#### *Congenital infection*

Congenital infection may occur either after a primary infection or a recurrent episode during pregnancy. Primary infection is more likely to result in transmission to the fetus, and the sequelae are likely to be more severe. In CMV-seronegative women who seroconvert during pregnancy, the rate of intra-uterine transmission is 35–40% compared with 0.2–2% in seropositive women.<sup>2</sup> On average, up to 1% of infants are infected with CMV *in utero*.<sup>3</sup> The age of the mother is another factor in transmission risk; up to 30% of women in their mid-teens shed CMV in the genital tract, but this figure declines steadily with age until levels are undetectable in women over 30 years old.<sup>4</sup>

#### *Postnatal infection*

Between 8% and 60% of infants are infected in the first 6 months of life through intrapartum, peri- and postnatal or breast milk transmission.<sup>5</sup> The rate of transmission is particularly high among small children in nursery schools and day-care centres;<sup>6</sup> approximately 80% of children aged 12–18 months shed CMV.<sup>7</sup>

Acquisition of CMV is related to socioeconomic conditions; up to 80% of children from developing countries and poorer areas of developed countries become infected by the time they reach puberty.<sup>8</sup> For middle-class communities in developed countries, the corresponding figure is approximately 50%.<sup>9</sup>

The prevalence of CMV infection in adult populations therefore varies widely in different parts of the world, age groups and socioeconomic groups.

Horizontal transmission of human CMV (HCMV) occurs through close physical contact with body fluids; sources of virus include oropharyngeal secretions, urine, cervical and vaginal excretions, semen, breast milk, tears, faeces and blood. Besides salivary shedding, CMV is also sexually transmitted.<sup>10</sup> Virus shedding from infected individuals can often be prolonged, and may last for months or even years.

## General Aspects of Cytomegalovirus Infection

In common with other herpesvirus infections, primary infection with CMV may be followed by either recurrent or chronic viral infection. CMV remains in a latent state in a variety of tissues including the salivary glands, monocytes and leucocytes. While recurrences and persistent virus excretion often result from reactivation of latent virus, re-infection with a different strain of CMV sometimes occurs with a genetically different virus. This is most commonly seen in the immunocompromised individual. Most CMV infections in the otherwise healthy host are asymptomatic; serious disease associated with the infection occurs mainly in individuals who have an immature or impaired immune system, such as newborns, individuals with HIV infection or individuals immunocompromised due to therapy required to prevent allograft rejection.

The clinical, virologic and immunologic features of primary CMV infections in the immunocompetent adult are shown in Figure 1.<sup>11</sup>

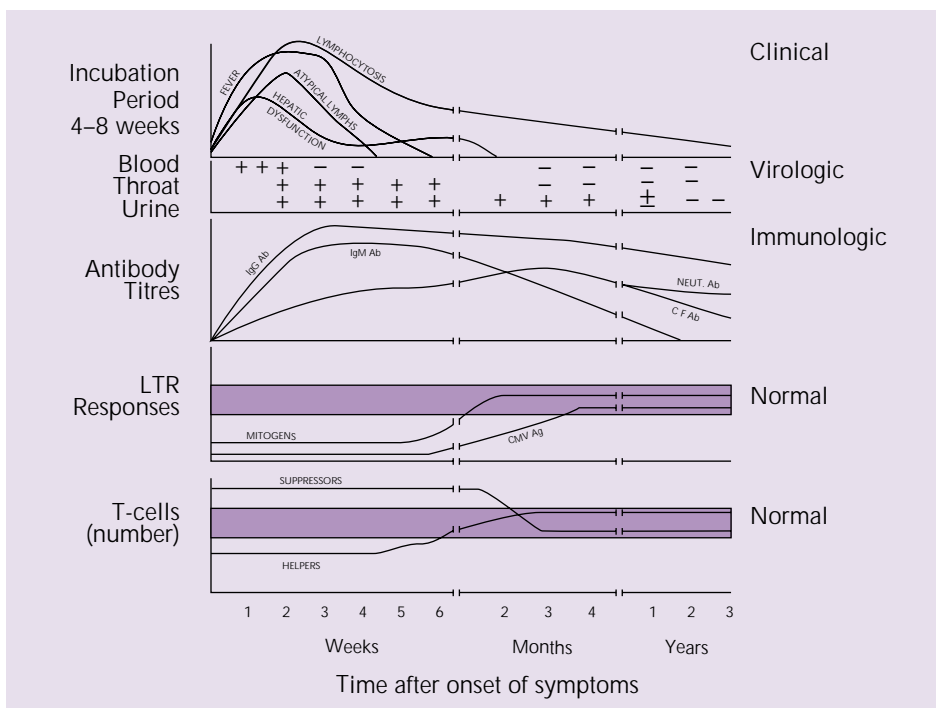


Figure 1: Features of CMV infection in the immunocompetent adult. Ab, antibody; CF Ab, complement fixation antibodies; LTR, lymphocyte transfer reaction; CMV Ag, CMV antigens<sup>11</sup>

The host immune response controls virulence during CMV infection and is a major factor in controlling and maintaining latency in CMV seropositive individuals. It is not surprising, therefore, that the virologic and immunologic events observed during CMV infection in immunocompromised individuals are exaggerations of those observed in the immunocompetent host.

### Clinical manifestations in the immunocompetent host

In the immunocompetent adult, primary CMV infection is usually asymptomatic but can result in a mononucleosis syndrome characterized by malaise, lymphadenopathy, pharyngitis, fever, myalgia, liver function abnormalities and lymphocytosis. The clinical course of the infection is usually mild, although a small percentage of patients suffer from protracted and severe fever.

The immunocompetent child infected with CMV may develop hepatitis accompanied by hepatomegaly, although the child may otherwise appear to be well.<sup>12</sup>

## *Manifestations of congenital infection*

Although CMV disease occurs more frequently and is more severe with congenitally acquired CMV infection, infection is most often subclinical. Intra-uterine, perinatal or early postnatal acquisition of CMV often leads to prolonged excretion of virus compared with acquisition of infection later in life. Virus is continuously shed in urine for as long as 5 years and into the nasopharynx for 2–4 years. Further, the quantity of virus shed at these sites is also much greater than that detected in older individuals.

CMV disease in the newborn is characterized by involvement of the reticuloendothelial and central nervous systems (CNS), and may include eye and auditory damage.<sup>13</sup> Signs observed include petechiae, hepatosplenomegaly, jaundice, microcephaly, intracranial calcifications and retinitis. Laboratory abnormalities are found in 50–90% of the cases, and mortality can be as high as 30%. However, many cases of fetal infection have minimal signs which may be undiagnosed but can lead to significant CNS damage in later years.<sup>14</sup>

Subclinical fetal infection has a much better prognosis; however, up to 10% of infants may develop severe and progressive deafness in the first 2 years of life.<sup>15</sup>

## *Clinical manifestations in the immunocompromised host*

In the immunocompromised host, primary CMV infection, reactivation and re-infection are all associated with significant morbidity and mortality. The clinical presentation of CMV infection in immunocompromised hosts varies according to the degree of immunocompromisation and to host factors, some of which are poorly understood. Populations at greatest risk of CMV infection and disease include individuals with HIV infection, transplant recipients, cancer patients receiving chemotherapy and burn patients. Manifestations of CMV disease in transplant recipients and HIV-positive individuals are listed in Table 1.

## Immunobiology of Cytomegalovirus Infection

### *Role of macrophages and endothelial cells in CMV persistence*

A major source of CMV in seropositive individuals is peripheral blood mononuclear cells (PBMC). Early epidemiological studies of bone marrow transplant (BMT) patients have shown that virus is transmitted to patients through transfusion of the leucocyte fraction of the peripheral blood.<sup>16</sup> Although only low percentages of CMV-infected cells are detected in the peripheral blood, transplanted organs exhibit a high frequency of infected leucocytes.<sup>17</sup> These observations suggest that peripheral blood cells are not only infected at a higher frequency but also that they are a potential reservoir as well as a vector for dissemination of CMV into target tissues.

Examination of peripheral blood cell populations from CMV-seropositive individuals has identified monocytes as the predominant infected cell-type.<sup>18</sup> Although CMV can infect monocytes the infection is nonproductive. However, when activated T-cells come in contact with infected monocytes the cells differentiate into macrophages which induce the virus to replicate and produce infectious CMV.<sup>19</sup> Monocyte–T-cell

**Organ transplant recipients HIV-positive individuals\***

<b>Overall CMV disease</b>	20–40%	50–70% at autopsy
<b>CMV manifestations</b>		
Pneumonitis	5–15%	?
Fever/constitutional symptoms	20–30%	?
Gastrointestinal tract	10–20%	5–15%
Hepatitis	10–50%	5%
Retinitis	<1%	15–40%
CNS	<1%	10–15%

\*CD4 <50 cells/mm<sup>3</sup>.

Table 1: Comparison of clinical manifestations of CMV disease in different groups of immunocompromised patients

interactions commonly occur during cellular immune responses that follow allograft transplantation. CMV infection of macrophages is non-cytopathic and results in a significant delay in the appearance of infectious virus (5–7 days post-infection [5–7 dpi]) in comparison to fibroblasts (2 dpi), the prototypic cell for growth *in vitro*. The delay in viral expression may be a unique adaptation of CMV to persist in the macrophage without the increase in viral factors which may be toxic to the cell.<sup>20</sup> Another mechanism that the virus utilizes to persist in the macrophages is the ability of

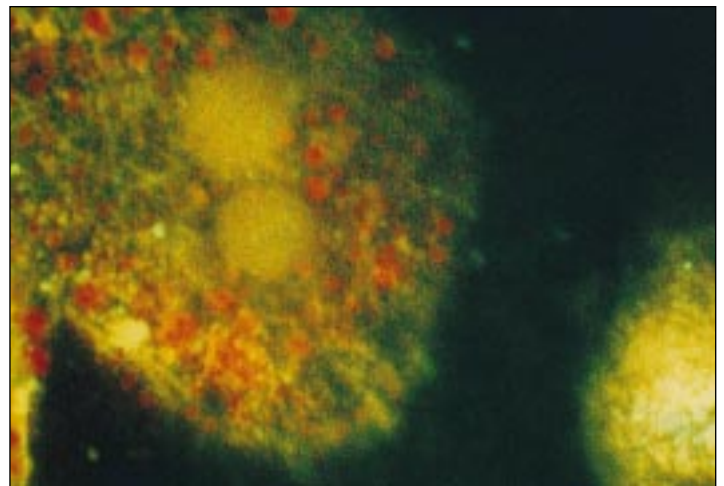


Figure 2: Confocal micrograph showing the accumulation of virus-infected vacuoles (red) in macrophages

CMV to accumulate in vacuolarized cytoplasmic compartments without reaching the surface of the cell (Figure 2).<sup>21</sup> Therefore, as macrophages traffic to tissues and fuse with target cells, virus can be delivered during the fusion process, virtually undetected by the immune system. The replicative stages of CMV infection in a macrophage and the dissemination of the virus into target tissues is depicted in Figure 3.

Another potential site for CMV persistence is vascular endothelial cells. Viral infection of endothelial cells is common in the organs of individuals with acute disease (Figure 4).<sup>22, 23</sup> The presence of CMV has also been detected in the aortic vessel walls of asymptomatic seropositive individuals.<sup>24</sup> Since the virus appears to be associated with atherosclerotic plaques, this observation has led to speculation that CMV is associated with coronary artery restenosis. In support of this hypothesis CMV has been associated with the development of restenosis, a disease which is characterized by smooth muscle proliferation similar to atherosclerosis.<sup>25</sup>

Endothelial cells in different organs not only serve different functional roles but also exhibit unique morphological and biochemical properties. For example, brain

# Chapter 1

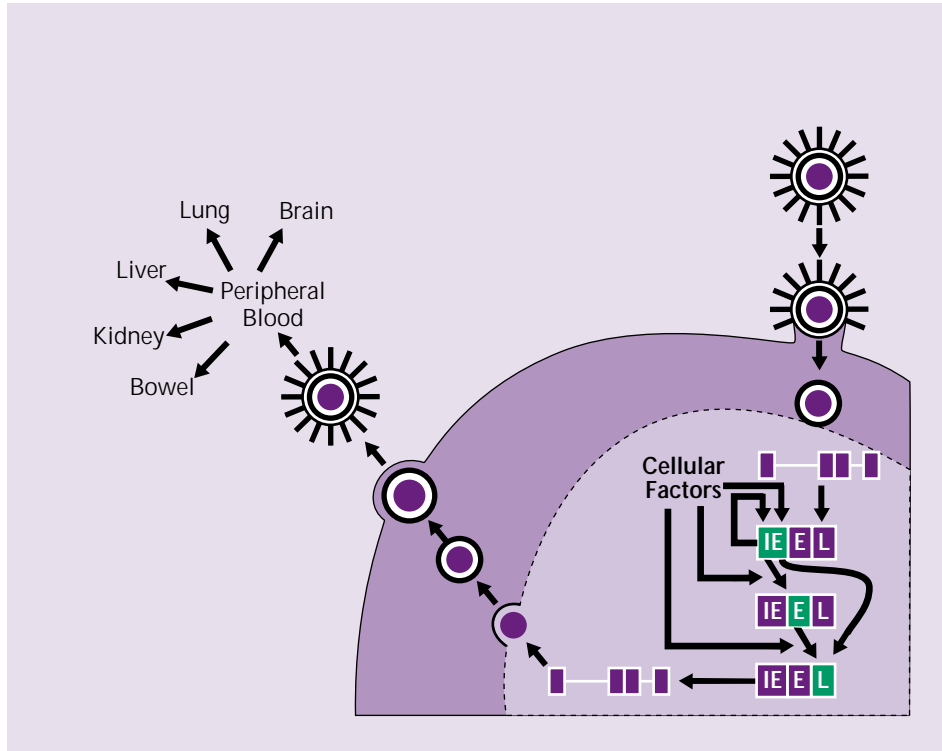


Figure 3: Diagram showing CMV spread. IE, immediate early; E, early; L, late

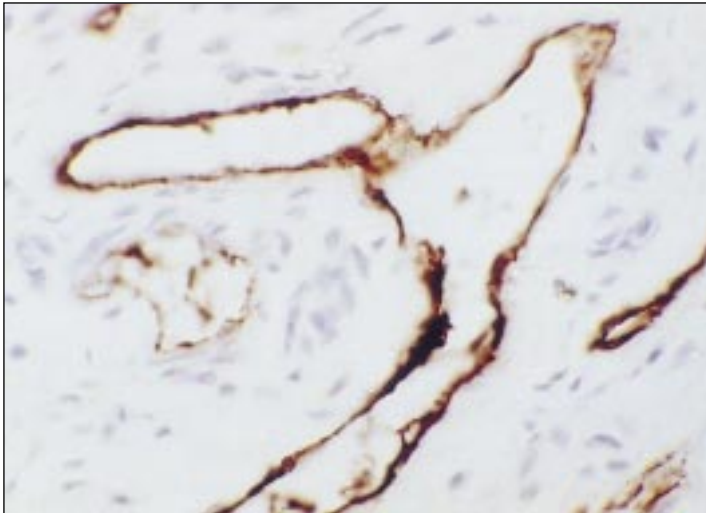


Figure 4: In situ hybridization of endothelial cells

microvascular endothelial cells which are a component of the blood–brain barrier are phenotypically and biochemically distinct from large vessel endothelial cells (i.e. aorta or umbilical). These unique cellular properties are exemplified by the replicative properties of CMV in these cells. CMV infection of brain endothelial cells results in the production of significant quantities of virus and lysis of the cell. In contrast, CMV infection of aortic endothelial cells results in chronic virus production without cellular cytopathogenic effect.<sup>26</sup>

Although the cell type(s) which are the latent or persistent site of CMV are unknown, both macrophages and endothelial cells are primary candidates. Since endothelial cells form the interface between the peripheral blood and the underlying tissues, dynamic interactions between these cell types may mediate the processes of persistence and dissemination which are a hallmark of CMV pathogenesis. Two potential models are shown in Figure 5, which depicts CMV infection and activation in these endothelial cells and macrophages which maintain persistence. In the first model, CMV-infected macrophages may infect activated endothelium as the macrophage traverses the vessel wall. The second model shows how CMV activated in endothelium during inflammatory reactions may infect macrophages trafficking through the vessel. These interactions appear to be key in the immunopathogenesis of CMV.

### Mechanisms of CMV-induced disease

CMV is one of the most common and important pathogens in the transplant setting. Clinical symptoms vary depending on the type of transplant. For example, in solid organ allograft recipients (except heart–lung transplants), severe infections of the gastrointestinal tract, leukopenia and thrombocytopenia are the most common manifestations of disease, while pneumonia is rare. However, in BMT and heart–lung recipients, pneumonia is the most common sequela and is often fatal.<sup>27,28</sup> Interestingly, while retinitis is rare in transplant patients, this clinical manifestation is among the most common sequela in individuals with AIDS.<sup>29</sup> The tropism of CMV for different tissues more commonly in different transplant contexts or other disease situations is unknown.

CMV can cause disease by a variety of different mechanisms. These include:

- ◆ Direct tissue damage
- ◆ Immunologic damage
- ◆ Indirect mechanisms

Similar to other viral pathogens, CMV can infect cells causing cellular death and ultimately tissue damage. Classically, CMV is primarily associated with infection of ductal epithelial cells especially in the salivary glands, bile duct and kidney. However, the virus can also infect mucosal epithelium of the gastrointestinal tract, where CMV can cause a necrotizing deep ulceration, as well as bladder epithelium where virus is subsequently shed in the urine. Mucosal epithelium of the respiratory tract is also another target for CMV infection. Although rare in transplant patients, CNS disease is common in individuals with AIDS as well as with congenital infection. While focal lesions are more common in congenital infection, individuals with AIDS more frequently exhibit diffuse encephalopathies in which ependymal, subependymal and deeper regions are affected.<sup>30</sup> An example of the latter disease process is demonstrated in Figure 6.

While direct infection of mucosal epithelial cells in the lung is a potential mechanism for organ damage and respiratory failure, animal models have suggested that immunologic destruction of the lung by the host immune response to CMV infection may be the major mechanism of viral disease in this tissue.<sup>31</sup> This hypothesis is supported by the observation that patients with CMV exhibit pronounced lung

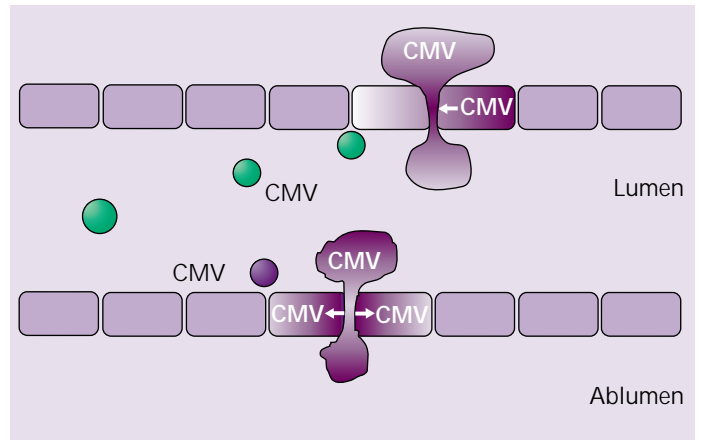


Figure 5: Two models for the processes of CMV infection and activation

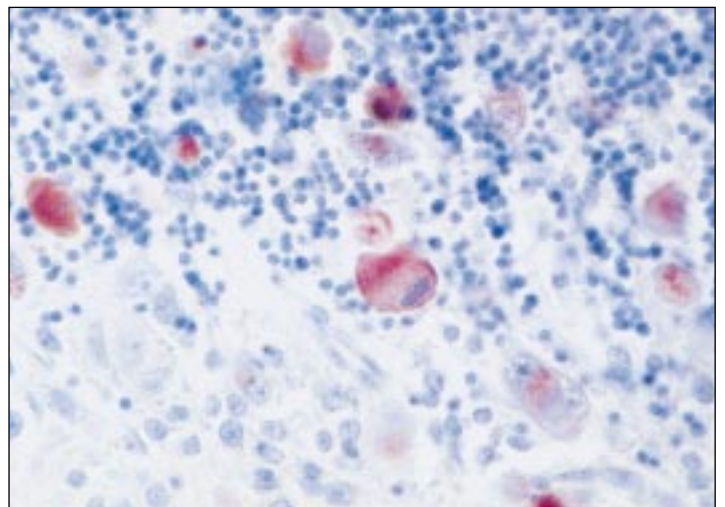


Figure 6: CMV encephalitis

insufficiencies which cannot be accounted for by the amount of viral infection in tissues.<sup>32</sup> While cytolytic T-lymphocyte (CTL) activity may contribute to this process, cytokines released by these cells have also been implicated. In addition, CMV may also directly stimulate the production of cytokines such as TNF- $\alpha$  and IL-1 which may contribute to cellular toxicity. Another potential immunologic mechanism of CMV-induced disease may occur in chronic graft versus host disease (GVHD). CMV has been directly linked to this disease process. Recent evidence has shown that CMV incorporates the cellular CD13 molecule into its virion.<sup>33</sup> CD13 in the context of the virion is associated with the induction of autoantibodies in GVHD.<sup>34</sup>

Finally, CMV has also been implicated in having a causal role in disease mechanisms. These disease processes are primarily exemplified in chronic organ rejection or transplant vascular sclerosis. This disease manifestation occurs in 30–50% of all allografts by 5 years post-transplant.<sup>35</sup> Histopathologically, chronic rejection is characterized by the development of vascular proliferation of smooth muscle cells which migrate from the media to the intima of the vessel. This disease is similar to atherosclerosis which is also associated with CMV infection. CMV infection is hypothesized to cause this disease through either direct infection of the smooth muscle cells which directly induces proliferation or infection of cells adjacent to the lesion. In the latter scenario the virus is thought to induce growth factors which contribute to the proliferative process.

In summary, CMV is the most important viral pathogen in transplant patients and one of the leading causes of death in individuals with AIDS. The virus has developed unique strategies to utilize cells of the immune system to traffic to target tissues in the immunocompromised host as well as multiple mechanisms to cause disease. Understanding viral replicative strategies and identifying cellular reservoirs will be important in the development of new antiviral therapies.

## Diagnosis and Quantification of Cytomegalovirus

An early diagnosis of CMV infection is desirable for the patient, as it will identify patients at greatest risk of disease and allow pre-emptive therapy to be initiated promptly. Conversely, a negative CMV test result can prevent the patient from undergoing unnecessary therapy with the attendant risk of any side-effects.

A comparison of common laboratory methods for detecting CMV is shown in Table 2. Conventional cell culture (CCC) detects CMV when viral replication has proceeded sufficiently to damage the cells (cytopathic effect). A more rapid method of detecting virus-infected cells is to stain them with monoclonal antibodies (Detection of Early

Method	Sensitivity	Specificity	Reliability	Rapidity	Prognostic value
CCC	++	+++	++		++
DEAFF	+	+++	++	++	++
LAD	++	+++	++	+++	+
PCR	+++	+++	++	++	++

CCC, conventional cell culture; DEAFF, detection of early antigen fluorescent foci, also known as the shell vial assay; LAD, leucocyte antigen detection; PCR, polymerase chain reaction.

Table 2: HCMV detection in body fluids

Antigen Fluorescent Foci [DEAFF] or shell vial assay). Different monoclonal antibodies can be used to detect CMV antigens circulating within peripheral blood leucocytes (leucocyte antigen detection). Finally, CMV DNA extracted either from whole blood or from plasma can be amplified using the polymerase chain reaction (PCR). Alternatively, virion mRNA can be extracted from leucocytes, reverse transcribed (RT) into DNA and then amplified (RT-PCR).

Each of these methods has benefits, but the most sensitive is the PCR technique, which is becoming increasingly recognized as a valuable prognostic tool.<sup>36,37</sup> An outline of how PCR works is shown in Appendix 2.

PCR can be applied to a variety of specimens to detect CMV DNA, including whole blood, plasma/serum and PBMC/DNA extracts. In addition, RT-PCR of PBMC RNA can be used to quantify CMV DNA.<sup>38</sup> Several of these methods have been shown in clinico-pathological analyses to be superior to standard methods (Table 3).

Assay	Sensitivity	Specificity	PPV	NPV
<b>Urine:</b>				
CCC	0.5	0.85	0.41	0.89
DEAFF	0.35	0.86	0.37	0.86
PCR	0.6	0.71	0.32	0.89
<b>Blood:</b>				
CCC	0.25	0.93	0.5	0.82
DEAFF	–	0.97	–	0.77
PCR	0.8	0.86	0.62	0.94

PPV, positive predictive value; NPV, negative predictive value.

Table 3: The sensitivity, specificity and predictive values for CMV disease associated with different detection methods for CMV in urine and blood<sup>36</sup>

	Plasma PCR	Leucocyte culture
● Sensitivity	97	73
● Specificity	58	64
● PPV	60	57
● NPV	97	78

Table 4: Correlation between detection of CMV and disease in BMT recipients<sup>39</sup>

Plasma PCR effectively identified infection and disease in 83 BMT patients, and showed improvements in sensitivity and predictive values over leucocyte culture (see Table 4).<sup>39</sup>

Patel *et al* (1995) reported similar findings; 41 liver transplant recipients were investigated for symptomatic CMV infection using PCR of serum and PBMC, RT-PCR of PBMC and viral blood culture.<sup>40</sup> While viral blood culture was the best technique for the *diagnosis* of symptomatic CMV infection, serum PCR was the most useful for predicting the *development* of symptomatic CMV disease.

## Risk Factors for Developing Cytomegalovirus Disease

The risk of a patient developing CMV disease depends on a number of factors, including:

- The CMV serostatus of the donor and recipient in transplant patients (Table 5)
- The CMV serostatus of the donor and recipient in blood transfusions
- The degree of immunosuppression. This influences reactivation of CMV
- The presence of CMV in the blood (viraemia), which is associated with a high risk of current or future disease
- The quantity of CMV (viral load)

- **D<sup>-</sup>R<sup>-</sup>** Donor negative, recipient negative
- **D<sup>+</sup>R<sup>-</sup>** Donor positive, recipient negative
- **D<sup>-</sup>R<sup>+</sup>** Donor negative, recipient positive
- **D<sup>+</sup>R<sup>+</sup>** Donor positive, recipient positive

Table 5: Combinations of donor (D) and recipient (R) serostatus

### CMV serostatus and degree of immunosuppression

In liver and renal transplant recipients, patients receiving organs from donors who are seropositive are at increased risk of CMV disease. In contrast, the increased risk of CMV disease in BMT recipients is associated with reactivation of recipient virus. There is some degree of protection against CMV disease if both the donor of T-cell depleted marrow and recipient are seropositive, indicating adoptive transfer of immunity from donor to recipient.

Patients are also at increased risk for CMV disease if they receive poorly matched organs and more intense immunosuppression. A study by Pass *et al* (1980), showed that renal transplant patients were more likely to develop CMV disease if they had received cadaveric organs, received antithymocyte globulin (ATG), or were viraemic.<sup>41</sup> Hanto *et al* (1994) compared antilymphocyte globulin (ALG) with OKT3 in cadaveric renal transplant recipients.<sup>42</sup> The authors found that the rates of patient survival and graft survival were similar in both groups. However, there were significant reductions in the incidence of CMV infection in OKT3-treated patients, whether the recipient was seronegative or seropositive. In donor positive, recipient negative (D<sup>+</sup>R<sup>-</sup>) patients, 8/10 patients in the ALG group developed CMV infection (six of whom had severe or moderate CMV disease) compared with only 2/15 patients receiving OKT3 ( $P=0.002$ ). Similarly, in D<sup>+</sup>R<sup>+</sup> patients 8/23 patients receiving ALG developed CMV infection (with five of these having moderate or severe disease), compared with 1/21 patients receiving OKT3 ( $P=0.02$ ).

In lung and heart–lung transplant recipients, patients in whom the donor is CMV seropositive are at substantially increased risk of obliterative bronchiolitis.<sup>43</sup> Up to 90% of patients in these groups who have cell culture evidence of CMV infection develop disease. Interestingly, bronchoalveolar lavage (BAL) from these patients contains primed lymphocytes, suggesting that obliterative bronchiolitis is an immunologically-mediated event representing chronic rejection.

In heart transplant patients, biopsies that were PCR positive for CMV DNA from 88 patients were most frequent in the D<sup>+</sup>R<sup>+</sup> group in association with moderate or severe

rejection.<sup>44</sup> Although there appears to be a definite association between rejection and CMV disease, the temporal relationship between the two events is not clear.

Similar findings have been observed in BMT patients. The use of T-cell depleted bone marrow (TCDM) is effective for the prophylaxis of GVHD, but in a study by Bunjes *et al* (1995), reactivation of virus in TCDM recipients led to more CMV infection than in patients receiving normal marrow and treated with cyclosporin and methotrexate.<sup>45</sup> Early infection in the TCDM group correlated with an early increase in blood lymphocytes (both natural killer and CD8 cells). This study suggests that the early immune responses to CMV may be associated with a more favourable clinical outcome.

Patients at greatest risk of transfusion-acquired CMV infection are those with an immature immune system or those who are immunosuppressed. For CMV-seropositive patients receiving blood from seropositive donors, the risk of CMV infection varies from 5% to 67%, with an average of 14%.<sup>46</sup> Infection rates with transfusion in infants are only slightly increased over those in older individuals, but with primary infection in premature babies, CMV-induced disease is much more common. Between 50% and 90% of the seronegative infants develop various forms of CMV-induced disease and mortality rates can range as high as 40%.<sup>46</sup>

In most western countries, 40–60% of blood donors are seropositive for CMV. Removal of leucocytes, which are assumed to be the site of latent CMV, has been suggested to prevent transfusion-acquired infection.<sup>47</sup> A multicentre, controlled trial in Australia was conducted to determine whether removal of leucocytes from blood by means of 'Imugard IG500' (Terumo) filters would prevent transfusion-acquired CMV infection in newborn infants.<sup>48</sup>

Seventy-two low birthweight infants whose mothers were seronegative and who received some seropositive blood were followed for 6 months for evidence of CMV infection. Although there were no significant differences between the groups who received filtered or unfiltered blood in median gestation, birthweight or amount of seropositive blood received, nine (21%) of the 42 infants who received unfiltered blood were infected with CMV compared with none of the 30 infants who received filtered blood. These results suggest that transfusion-acquired CMV infection is preventable by filtration of blood through a leucocyte filter. However, the threshold leucocyte number at which disease manifests itself is still to be determined.

### *Presence of CMV in body fluids*

There is evidence that the quantity of CMV in body fluids correlates with the likelihood of developing disease. Data from a study by Stagno *et al* (1975) which measured CMV in the urine of congenitally-infected infants show that high viral load is associated with a poor prognosis.<sup>49</sup> Patients with visceral disease and BMT recipients have higher viral loads of CMV in their blood, while renal transplant recipients have high CMV levels in their urine.

In renal transplant recipients with symptomatic CMV disease, PCR analysis of urine has revealed high viral loads.<sup>50</sup> Conversely, asymptomatic patients have lower peak viral loads (Figure 7).

# Chapter 1

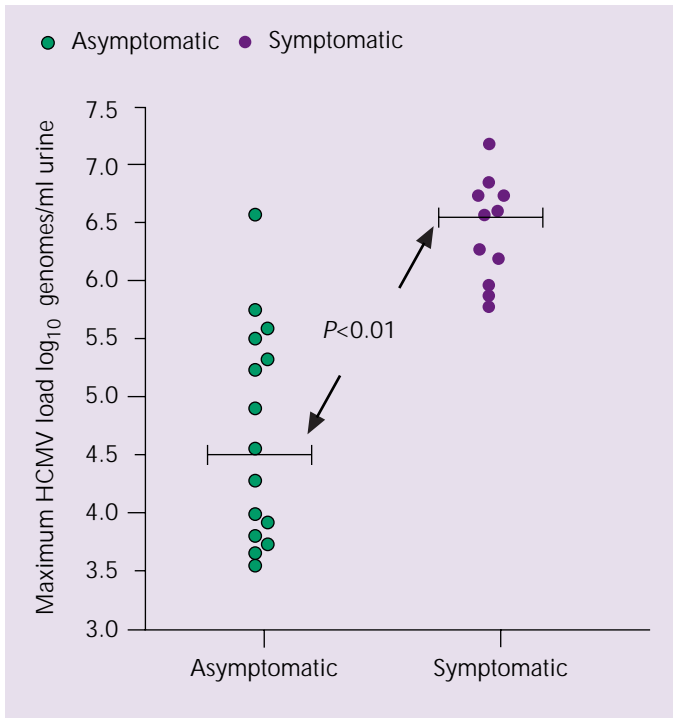


Figure 7: Correlation of viral load with clinical disease

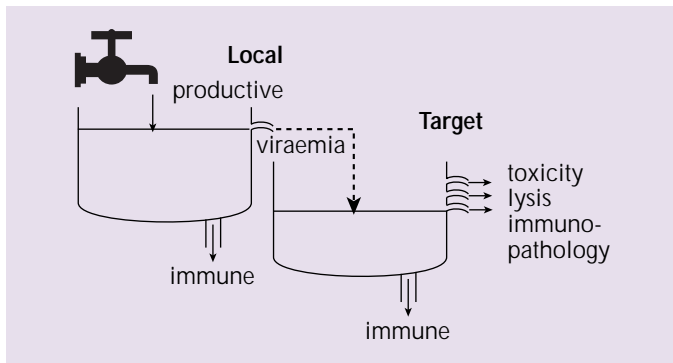


Figure 8: Schematic representation of the threshold theory of how viral replication may lead to pathological consequences

These data suggest that the probability of CMV disease increases dramatically when viral load reaches a certain threshold. A graphical representation of how certain thresholds of viral replication could lead to viraemic spread and pathology is shown in Figure 8. The analogy used is two baths which represent local and target organs for CMV replication. The tap represents production of virus, the plug hole, the immune system and the overflow, viraemic spread in the case of local replication or pathological manifestations of CMV replication in the target organ. In the context of the renal transplant recipient, local infection within the kidney could reach a level which exceeds the level controlled by the immune system and hence allows viraemic spread of infection to other target organs. Therefore, viraemia is associated with increased viral disease since virus will be disseminated to multiple target organs. Within the target organ, the immune system can again control the level of replication. If the threshold viral load is exceeded in this target organ then it may set in train a series of events leading to pathological consequences either through direct viral replication or through immunopathologic mechanisms.

## Summary and Management Recommendations

The categorization of management recommendations is outlined in Appendix 1.

*In vivo*, CMV interacts with several different cell types. In early disease, monocytes, macrophages and endothelial cells are involved, while epithelial cells have a role in late disease. Virus infection affects the function of the cells with which it interacts. Activation of macrophages, whether by transplant or by another infection, is critical for the dissemination of CMV.

Evidence from epidemiological and animal studies show that CMV has an immunosuppressive effect and increases the risk for other infections (e.g. secondary bacterial and fungal infections). The mechanism for this effect includes a complex interaction between CMV and host, with both direct effects and subsequent cytokine-mediated events. CMV has profound effects on individual cell types in the laboratory; this may be reflected in CMV-infected patients. The clinical manifestations vary markedly depending on the context of infection (i.e. type of transplant and type of immunosuppression), so potentially the benefits achieved by preventing CMV disease may extend beyond the benefits to other areas in the general health of the patient.

CMV disease is a significant problem in the transplant population. It is a major cause of death and graft rejection.

- ◆ As such, all transplant programmes should have policies for both surveillance and management strategies to detect or prevent CMV disease.
- ◆ Clinicians should choose between the various treatment strategies according to their access to laboratory diagnostic procedures and there should be close collaboration between diagnostic laboratories and clinicians to formulate monitoring and management strategies.

### *Category 3 Recommendation*

- ◆ Studies of CMV infection and disease in transplant patients should refer to the diagnostic criteria agreed in Paris and Stockholm.<sup>51</sup>
- ◆ All studies on CMV should define the criteria used for the diagnosis of clinical disease.

Blood product management and organ tissue typing should be used effectively:

- ◆ Ideally, all transplant recipients should be given CMV-negative or filtered blood. This will prevent infection in the seronegative patient and could prevent the acquisition of new strains of CMV in the seropositive patient. If cost becomes an issue, seronegative patients should be prioritized to receive filtered blood.

### *Category 3 Recommendation*

- ◆ BMT patients who are transplant candidates and who are CMV-negative should be maintained as CMV-negative by use of screened or filtered blood products.

### *Category 3 Recommendation*

- ◆ Lungs from CMV-positive donors should not be given to CMV-negative recipients assuming there is a choice and lungs from CMV-negative donors are available, because of the risk of long-term obliterative bronchiolitis.

### *Category 2 Recommendation*

Prophylaxis for CMV is not completely effective, so patients receiving prophylaxis should still be monitored for CMV infection. Blood is the preferred site for monitoring, but urine may be of value in the case of kidney transplant recipients. Regular sampling, preferably weekly, is recommended, especially in groups with a high risk of disease. Individual institutions should formulate their own guidelines for frequency of monitoring based on their centre's experience of CMV disease over time and individual patient needs.

In order to identify patients with CMV disease early and initiate pre-emptive therapy as soon as possible, sensitive detection methods are essential. Validated methods of CMV detection include cell culture, DEAFF, PCR and leucocyte antigen detection. Whichever method is used, it should be continually monitored for positive predictive values.

A standardized, commercially-available assay for detection of CMV is needed. Technologies for detection currently vary between centres. Existing tests should be audited frequently.

#### *Research need*

- ◆ The shell vial (DEAFF) assay is still useful; for example, it is good for detecting CMV in BAL. Because the test is not very sensitive, it gives positive results in patients with highest viral load, so is useful for prognosis. The shell vial assay is no longer appropriate for testing blood, especially if patients are receiving prophylaxis.
- ◆ The antigenaemia test has been utilized very effectively in many transplant centres, but can be subject to reader expertise.
- ◆ As yet, there is no commercial, licensed PCR test. However, PCR should be considered for diagnosis of CMV infection of the CNS by using cerebrospinal fluids.
- ◆ It is recommended that clinicians work with their local virologists to audit the usefulness of assays for CMV detection and to compare new assays for CMV with existing assays for both diagnostic and prognostic information. Such approaches will enable comparisons between centres for the prognostic values associated with different test methods.

High viral loads cannot at present provide prognostic information, although they are temporally associated with disease.

- ◆ Methods should be developed which are able to predict future high viral loads rapidly and reliably.

#### *Research Need*

These principles may apply to all immunocompromised patients and should be evaluated in other groups, e.g. cancer patients.

## Management of Cytomegalovirus Infection and Disease

### Choosing a Management Strategy for Cytomegalovirus

Choosing the best management strategy for an individual with cytomegalovirus (CMV) infection can often be difficult as several factors need to be considered; the individual's risk for CMV disease, the type of diagnostic tests available to the physician, and the likely outcome of CMV disease. Choice is further complicated by the confusion surrounding interpretation of clinical trials results; definitions of CMV disease versus CMV infection, different diagnostic techniques and treatment protocols which differ significantly between studies.

A high-risk group of patients with a poor expected outcome merits the use of a different strategy from that employed for patients with a low risk of disease and an anticipated good outcome. Most studies in the bone marrow transplant (BMT) setting involve high-risk patients with a poor outcome. Solid organ transplant recipients are generally at high risk of disease, but usually have a good outcome. Prophylaxis and pre-emptive therapy (Table 1) have mainly been used in these two contexts. Prophylaxis literally means 'treatment before disease'. In studies in transplant patients, prophylaxis usually refers to administration of a drug from the time of transplant onwards. Prophylaxis is different from pre-emptive or early therapy, in which treatment is initiated following the detection of CMV infection during routine surveillance and monitoring of the patient.<sup>1,2</sup>

<b>Prophylaxis</b>	Therapy given before any virologic evidence of CMV infection is present. The objective is to prevent CMV infection and disease, and prophylaxis is administered to all patients at risk
<b>Pre-emptive therapy</b>	Therapy given at the first virologic detection of CMV infection, before clinical disease has developed. Treatment is initiated after detection of virus in a normally sterile fluid or secretion; therefore, only a select group of patients will receive treatment

Table 1: Definitions of prophylaxis and pre-emptive therapy

In order to prevent transmission of CMV from a seropositive organ or blood donor, CMV-seronegative patients who receive seronegative solid organs or marrow should receive exclusively filtered blood products, or blood products from CMV seronegative donors.

In CMV seropositive patients or seronegative patients who receive an organ from a donor who is seropositive there are other options. Treatments can be divided into low potency drugs, such as aciclovir or immunoglobulins, and high potency drugs such as ganciclovir, foscarnet or cidofovir. Existing data suggest that immunoglobulins reduce the severity of primary CMV infection and CMV disease in solid organ transplant recipients, and are

reasonably well tolerated. However, immunoglobulin treatment is very expensive and its efficacy in BMT recipients remains controversial. In most situations, especially in seropositive individuals, antiviral prophylaxis is preferable to immunoglobulin prophylaxis.

## Treatment of Established Cytomegalovirus Disease

### Definitions of infection and disease

Definitions of infection and disease have been previously agreed at the *4th International CMV Conference (ICMVC)* in Paris in 1993 (Table 2)<sup>3</sup> and revised at the *5th ICMVC* in Stockholm in 1995.<sup>4</sup>

#### CMV Infection

- Positive culture
- Positive antigenaemia test
- Positive polymerase chain reaction (PCR) in blood or plasma
- Positive tissue specimen (culture, histology, immunochemistry, *in situ* hybridization)

#### CMV Disease

- Virologic evidence of infection in tissue specimens, PCR on cerebrospinal fluid or bronchoalveolar lavage fluid associated with appropriate clinical symptoms and signs, and histopathology to support diagnosis for liver and gastrointestinal disease

### Currently available antiviral drugs against CMV

Antiviral drugs can be effective in the prophylaxis and treatment of CMV infection and disease. Drugs used or investigated for the treatment of established CMV disease include ganciclovir, foscarnet and more recently cidofovir (HPMPC; (S)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl] cytosine), which has been evaluated mainly for the treatment of CMV retinitis in individuals with AIDS. The chemical structures of these drugs are shown in Figure 1.

Table 2: Definitions of CMV infection and disease

### Mechanisms of action

Nucleoside analogues such as ganciclovir need to be activated in CMV-infected cells before they are able to inhibit the viral DNA polymerase and thereby inhibit viral replication. The CMV UL97 gene product, which encodes a phosphotransferase, activates ganciclovir. Further phosphorylation steps are then catalysed by cellular enzymes, and viral DNA polymerase is directly inhibited by the corresponding nucleoside analogue triphosphate. Cidofovir is an analogue of a nucleoside monophosphate. It does not require activation by UL97 but is converted to its diphosphate by cellular enzymes.

Foscarnet is a pyrophosphate analogue which does not require activation within target cells, but acts directly on viral DNA polymerase by binding to the site where the pyrophosphate binds, preventing cleavage and incorporation of the nucleotide into the DNA chain. Mechanisms of action of these drugs are summarized in Figure 1.

## Treating Established Cytomegalovirus Disease in Bone Marrow Transplant Recipients

### CMV infection and disease in BMT recipients

Several studies performed in the early 1980s indicated that CMV pneumonia occurred in approximately 30% of all allogeneic BMT patients in the absence of antiviral drug

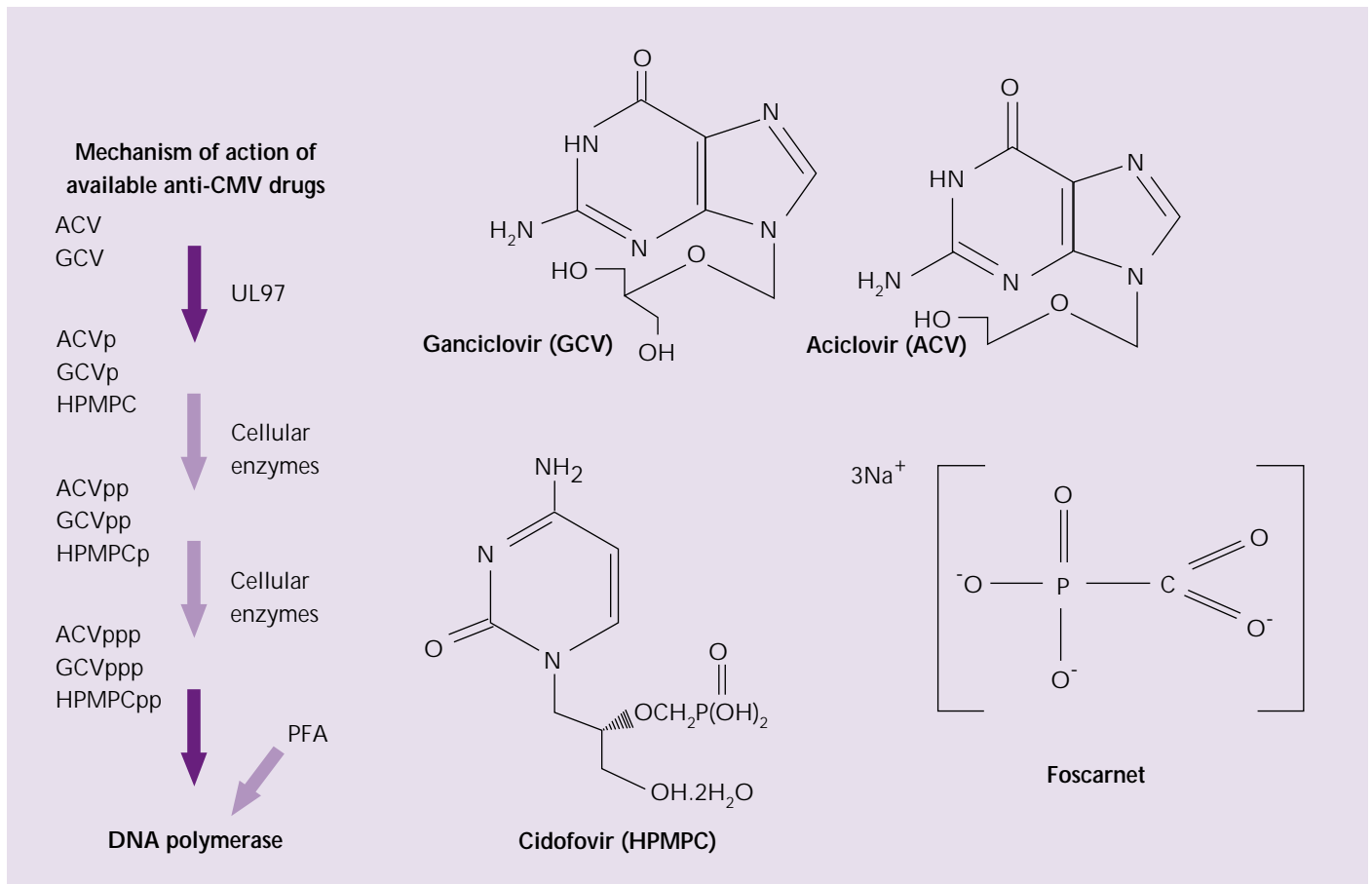


Figure 1: Chemical structures of drugs used in the prophylaxis and treatment of CMV disease and their mechanisms of action. PFA, phosphonoformacic acid; p, monophosphate; pp, diphosphate; ppp, triphosphate

prophylaxis. Recently, this frequency has decreased due to the increased use of prophylaxis and pre-emptive therapy. Once CMV pneumonia occurs, mortality is around 85% if untreated. In autologous BMT patients, who are less immunocompromised, there is a lower incidence of CMV pneumonia (around 4%). However, one study has shown that the case fatality rate in those autologous BMT patients who did develop CMV pneumonia was around 80%.<sup>5</sup>

### Antiviral drug monotherapy

The severe state of immunocompromisation in the BMT patient makes therapy of established CMV disease difficult. Antiviral drug monotherapy is not effective in the treatment of CMV pneumonitis.

Aciclovir was one of the earliest drugs to be studied as monotherapy in CMV disease. In one study, eight BMT patients with CMV pneumonia were treated with intravenous aciclovir 400 mg/m<sup>2</sup>–1200 mg/m<sup>2</sup> every 8 hours, with a similar outcome to that seen in untreated patients.<sup>6</sup>

Ganciclovir became available in the mid-1980s. While similar to aciclovir in chemical structure (Figure 1), *in vitro* data show that it is up to 100-fold more effective against CMV than aciclovir. Shepp *et al* (1985) showed that in 10 patients with biopsy-proven CMV pneumonia, ganciclovir had a clear antiviral effect; within 6–12 days virus shedding had stopped (Figure 2).<sup>7</sup> However, ganciclovir treatment did not

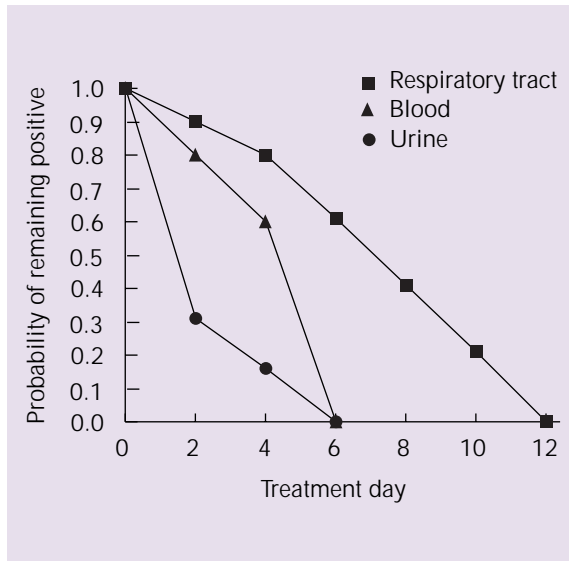


Figure 2: Elimination of CMV from cultures obtained during ganciclovir treatment<sup>7</sup>

prevent progression to respiratory failure and death in marrow transplant recipients with CMV pneumonia (only one of the 10 patients treated survived). It was also noted that marrow toxicity could complicate therapy at doses >3 mg/kg/day or in patients with moderate renal dysfunction.

Similar results were seen in another study with ganciclovir monotherapy. A placebo-controlled trial of ganciclovir in CMV gastroenteritis, studied 37 BMT patients who received either intravenous ganciclovir (2.5 mg/kg, every 8 hours for 14 days) or placebo for biopsy-proven gastrointestinal (GI) disease.<sup>8</sup> Ganciclovir reduced the rate of positive CMV cultures from throat, urine and oesophagus significantly compared with placebo. However, there was no difference in the clinical symptomatology or in the endoscopic appearance of GI lesions between the two groups after treatment.

Similarly, several studies also suggest that therapy with foscarnet alone is not effective for proven CMV disease in BMT recipients. A study in BMT patients at the Karolinska Institute using intravenous foscarnet at 9 mg/kg (bolus) followed by continuous infusion of 112 mg/kg/day showed that of the eight patients with pneumonia, all died; of the patients with pancytopenia two out of three died and one improved, one patient with hepatitis improved and one patient with encephalitis improved.<sup>9</sup> A subsequent study in 42 BMT patients showed similar results: all 15 patients with proven CMV pneumonia died.<sup>10</sup>

### Therapy with combinations of antiviral drugs

Various combination drug therapies for CMV pneumonia in BMT patients were generally ineffective.

Few studies have evaluated combinations of the newer antiviral drugs, e.g. foscarnet plus ganciclovir or ganciclovir plus cidofovir. Table 3 shows survival rates according to treatment.<sup>11</sup>

Drug regimen	Number of patients treated	Survival (%)
Vidarabine	9	22
Interferon	8	0
Vidarabine + interferon	7	14
Aciclovir	8	13
Aciclovir + interferon	21	19
Aciclovir + vidarabine	6	0
Ganciclovir	10	10
Ganciclovir + steroids	6	17
CMV immunoglobulin	14	21
<b>Average</b>		<b>15</b>

Table 3: Treatment of CMV pneumonia with antiviral monotherapy and combination therapy<sup>11</sup>

The average survival rate with the treatment modalities shown in Table 3 was 15%, which is similar to that in patients who received no treatment at all. More recently, non-comparative studies in small numbers of allogeneic BMT recipients were carried out in three transplant centres, using a combination of ganciclovir and high doses of CMV immunoglobulins. This resulted in an increased survival rate compared to historical controls who received no therapy (Table 4).

Combination therapy:	Study		
	Emanuel <i>et al</i> , 1988 <sup>12</sup> (n=10)	Reed <i>et al</i> , 1988 <sup>11</sup> (n=25)	Schmidt <i>et al</i> , 1988 <sup>13</sup> (n=13)
<b>Induction:</b>			
Ganciclovir (mg/kg)	2.5 q8h, 20d	2.5 q8h, 14d	5q12h, 21d
Ivlg (mg/kg)	500 qod, 20d	400 on d1, 2, 7; 200 on d14	500 qod for 21d
<b>Maintenance:</b>			
Ganciclovir (mg/kg)	5 on 3–5 d/wk (20x)	5/d for 14d	5 on 5d/wk
Ivlg (mg/kg)	500 2x/wk (8x)	200 on d21	500 1x/wk
<b>Survival n (%)</b>	7 (70%)	13 (52%)	9 (69%)

Table 4: Combination therapy with ganciclovir plus intravenous immunoglobulins (Ivlg) of CMV pneumonia in BMT recipients<sup>11–13</sup>

Patients receiving this combination therapy were followed up at 6 weeks and again at 6 months after the start of treatment. At 6 weeks, the proportion of patients surviving was quite high; however, after 6 months the survival rate was considerably lower (Table 5). In a later study from the European Group for Blood and Marrow Transplantation (EBMT) the success rate was already lower at 6 weeks.<sup>14</sup>

The overall mortality in patients who developed CMV pneumonia early after transplant and who survived following therapy with ganciclovir plus immunoglobulins, was compared with the mortality in those patients who never developed CMV pneumonia in the post-transplant course. There was a trend towards a lower survival rate for patients who developed CMV pneumonia and survived. When non-leukaemic mortality was assessed in these two patient groups, the difference between the two groups was even more pronounced and approached statistical significance. In the patients who survived CMV pneumonia, a high proportion died from fungal infections and gram-negative septicaemia; the precise reasons for the difference in outcome between the two patient populations remain unclear.

	Survival at	
	6 weeks	6 months
◆ Emanuel <i>et al</i> , 1988 <sup>12</sup>	65%	30%
◆ Reed <i>et al</i> , 1988 <sup>11</sup>	48%	38%
◆ Schmidt <i>et al</i> , 1988 <sup>13</sup>	84%	40%
◆ Ljungman <i>et al</i> , 1992 <sup>14</sup>	31%	22%

Table 5: Long-term survival from CMV pneumonia treated with ganciclovir plus intravenous immunoglobulins after BMT<sup>11–14</sup>

## Treating Established Cytomegalovirus Disease in Solid Organ Transplant Recipients

### *Epidemiology of CMV disease in solid organ transplant recipients*

For renal transplant recipients who receive no prophylaxis and who are seropositive for CMV or who have a seropositive organ donor, the incidence of CMV infection is 40–70% during the first 3–4 months after transplant and the incidence of symptomatic CMV disease is about 20%.<sup>15,16</sup>

### *Antiviral drug monotherapy of CMV disease in solid organ transplant recipients*

Unlike the BMT patient, some success has been reported from uncontrolled trials for the use of antiviral drug monotherapy in solid organ transplant patients. Table 6 summarizes various studies in this area.

The studies summarized in Table 6 show that CMV disease responds in most cases to intravenous ganciclovir therapy alone in various solid organ transplant populations, including kidney, liver, heart and heart–lung transplant recipients.

Although there are limited data available for treatment of CMV disease with foscarnet in solid organ transplant recipients, one small study by Ringdén *et al* (1986) of renal transplant recipients treated with foscarnet yielded encouraging results (Table 6).<sup>9</sup>

### *Therapy with combinations of antiviral drugs in solid organ transplant recipients*

There are no published data on the clinical use of combinations of drugs in organ transplantations.

However, data from an *in vitro* study investigating the combination of foscarnet and ganciclovir against several strains of CMV showed that there may be a synergistic effect between the two drugs. In this study, the IC<sub>50</sub> of foscarnet alone was 133.0 µM, but as ganciclovir was added at increasing concentrations, the IC<sub>50</sub> of foscarnet was substantially reduced. The IC<sub>50</sub> of ganciclovir alone was 9.0 µM, but was similarly reduced by the addition of foscarnet.<sup>27</sup> The results from this study indicate that a synergistic effect between the two drugs exists, and strategies using the two drugs together may lead to an improved clinical benefit. Foscarnet and ganciclovir have been used together successfully to treat CMV disease in HIV-infected individuals.

## Results with New Antiviral Therapies

In the past few years several new antiviral agents have been investigated with the aim of extending the range of options available to treat CMV disease.

A recent study by Lalezari *et al* (1995) in HIV-positive individuals investigated the anti-CMV effect of cidofovir, and showed that treatment with this drug led to a substantial decrease in CMV excretion in semen and urine in a dose-dependent manner.<sup>28</sup> Although cidofovir has been associated with nephrotoxicity, the concomitant use of probenecid and prehydration before cidofovir therapy can substantially reduce renal toxicity.

## Current practice in the therapy of established CMV disease

CMV pneumonia in BMT recipients is currently being treated by the combination of ganciclovir and high doses of intravenous immunoglobulins. There are insufficient data available to make recommendations on therapy for other types of CMV disease in BMT patients. In solid organ transplant recipients, most centres are treating CMV pneumonia with intravenous ganciclovir alone for 2–4 weeks. Other CMV disease in these patients is treated for 2 weeks with ganciclovir monotherapy.

In conclusion, the results of drug therapy of established CMV disease after BMT are poor. Earlier intervention strategies in BMT recipients to prevent CMV disease are more promising. In solid organ transplant recipients antiviral drug therapy of CMV disease appears more effective, but CMV drug prophylaxis may reduce the morbidity associated with CMV disease substantially.

## Prophylaxis

Prophylaxis with low potency drugs such as aciclovir, or intravenous immunoglobulin, is associated with relatively less toxicity and may, in some patient populations, be beneficial for preventing CMV disease by reducing CMV viral load below the threshold associated with disease. Intensive monitoring is essential if low potency prophylaxis is used, and there is a risk that CMV disease will develop before a positive test result is obtained. High potency prophylaxis with ganciclovir or foscarnet may be more appropriate in severely immunosuppressed patients.

### Prophylaxis in BMT recipients

#### Prophylaxis with low potency drugs

Aciclovir prophylaxis has been shown to reduce the risk of developing CMV disease in BMT recipients. In a non-randomized, double-blind, placebo-controlled study by Meyers *et al* (1988), 86 BMT patients who were seropositive for CMV before transplant were given intravenous aciclovir (500 mg/m<sup>2</sup> every 8 hours) from 5 days before transplantation to 30 days post transplant.<sup>29</sup> Patients who received aciclovir had a significantly reduced risk of developing CMV disease, accompanied by an increase in survival, aciclovir-treated patients having a relative risk of death of 0.4 compared with the control group.

Prentice *et al* (1994) investigated aciclovir therapy in BMT recipients. Patients were randomized to receive one of three drug regimens:<sup>30</sup>

- Group A** intravenous aciclovir (500 mg/m<sup>2</sup>, three times daily) for 1 month followed by oral aciclovir (800 mg four times daily) for a further 6 months (high-dose group)
- Group B** intravenous aciclovir followed by oral placebo (intermediate group)
- Group C** low-dose oral aciclovir (200 mg or 400 mg four times daily) followed by placebo (low-dose control group)

In the high-dose group, aciclovir reduced CMV infection and CMV viraemia, although there was no significant difference in CMV disease, compared with controls. However, there was a significant survival benefit of 11% in the high-dose aciclovir treatment arm. Figure 3 shows the survival benefits of aciclovir in this study.

Study	Patient population	Treatment regimen
Erice <i>et al</i> , 1987 <sup>17</sup>	(n=31) 15 BMT recipients 5 kidney transplants 1 liver transplant 6 individuals with AIDS 3 patients with haematological malignancies 1 patient with systemic lupus erythematosus	Ganciclovir 2.5 mg/kg 3x/day
Dunn <i>et al</i> , 1991 <sup>18</sup>	(n=93) Solid organ transplant recipients with tissue-invasive CMV disease	Ganciclovir 1.25 mg/kg q48h to 5 mg/kg q12h, depending on renal function
Snydman, 1988 <sup>19</sup>	(n=17) Renal transplant recipients	Ganciclovir 5 mg/kg q12h <i>or</i> 2.5 mg/kg q8h iv for ≤14d
Jordan <i>et al</i> , 1992 <sup>20</sup>	(n=36) Renal transplant recipients with tissue-invasive CMV disease	Ganciclovir 2.5 mg/kg q12h
Harbison <i>et al</i> , 1988 <sup>21</sup>	(n=12) 9 liver and 3 kidney transplant recipients	Ganciclovir 0.75–7.5 mg/kg/day for 10–30 days
Paya <i>et al</i> , 1988 <sup>22</sup>	(n=17) 12 liver, 5 kidney transplant recipients	Ganciclovir 7.5 mg/kg/day iv
Paya <i>et al</i> , 1989 <sup>16</sup>	(n=6) Liver transplant recipients	Ganciclovir 2.5 mg/kg q8h iv
Stratta <i>et al</i> , 1989 <sup>23</sup>	(n=69) Liver transplant recipients	Ganciclovir 5 mg/kg q12h iv for 14 days
Keay <i>et al</i> , 1988 <sup>24</sup>	(n=22) 16 heart and 6 heart–lung transplant recipients	Ganciclovir 4 patients died during initial therapy.
Cooper <i>et al</i> , 1991 <sup>25</sup>	(n=22) Heart transplant recipients with proven CMV disease	Ganciclovir
Cerrina <i>et al</i> , 1991 <sup>26</sup>	(n=21) Heart–lung and double lung transplant recipients	Ganciclovir 10 mg/kg/day iv for 16 ± 3 days
Ringdén <i>et al</i> , 1986 <sup>9</sup>	(n=11) Kidney transplant recipients	Foscarnet 9 mg/kg iv then 112 mg/kg/d for median of 14 days

## Outcome

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Clinical improvement in 17 patients (55%)  
11 patients developed neutropenia

83/93 patients responded to therapy (89%)

5 patients experienced toxicity. No patients died

12 patients with CMV pneumonitis: 4 improved and 8 died;  
2 patients with generalized CMV disease died

All patients clinically improved. 100% survival at 1 year

8/12 (67%) clinically stabilized or improved  
67% had mild neutropenia  
Overall survival 50%

10/17 were clinically evaluable; 1/10 died

5/6 patients had clinical and virologic cure

51/69 had a prompt and lasting response  
CMV disease ultimately controlled by ganciclovir in 94.2% of the patients

16/22 survivors (82%) improved  
4 patients had neutropenia

All patients successfully treated

20/21 (95%) survived initial episode of CMV disease

7/11 improved  
3/11 died  
1/11 remained unchanged  
3/4 patients with CMV pneumonia survived

Table 6: Studies of antiviral monotherapy of CMV disease in solid organ transplant recipients<sup>9,16-26</sup>

# Chapter 2

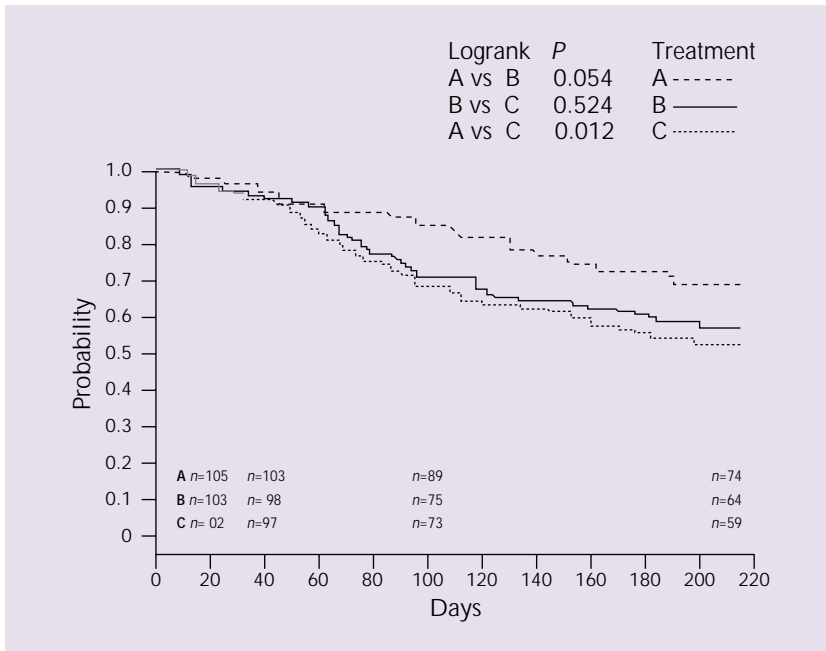


Figure 3: The survival benefits of aciclovir in a trial of BMT recipients<sup>30</sup>

The causes of death in this study showed that the mortality from CMV pneumonia was lower in the high-dose aciclovir arm (Table 7). There was also a lower rate of all infectious disease mortality (including all viral, bacterial and fungal infections). One of the factors underlying this result may be the immunosuppressant effect of CMV; reducing CMV infection with antiviral prophylaxis may also reduce the risk of other infections.

### Prophylaxis with high potency drugs in BMT recipients

Prophylaxis with the high potency drugs foscarnet or ganciclovir is effective for CMV disease prevention, although there may be

side-effects with these drugs, including neutropenia in ganciclovir-treated patients, and renal toxicity and electrolyte disturbances with foscarnet. There may also be an increased risk of late CMV disease if ganciclovir is given prophylactically over longer time periods. This may in some part be due to prophylaxis preventing the development

	Group A	Group B	Group C
CMV pneumonia	0	6	6
CMV hepatitis	0	1	0
CMV viraemia	1	0	1
Microbial pneumonia	2	1	9
Other microbial infections	5	11	10
Septic shock	1	2	2
Idiopathic pneumonia	6	7	6
Leukaemia	5	11	6
GVHD	5	7	3
Hepatic failure	2	1	2
Veno-occlusive disease	2	2	1
Renal failure	3	1	4
Cardiac failure	0	3	0
Adult respiratory distress syndrome	0	1	2
Other causes*	2	6	10
<b>Total†</b>	<b>26</b>	<b>38</b>	<b>42</b>

\* Includes haemorrhage, multisystem failure pulmonary fibrosis, pulmonary oedema, hypotension, neurological disorder, neoplasm and metabolic disorder.  
 † More than one cause was identified for some deaths.  
 GVHD, graft versus host disease.

Table 7: Number of deaths and causes<sup>30</sup>

of immune responses to CMV by maintaining viral load below a threshold level, and may also be a consequence of the prophylactic strategy itself; prophylaxis can only be effective while it is being administered. When prophylaxis is stopped, disease may develop, giving the appearance of delayed disease.

Two randomized, placebo-controlled studies of ganciclovir in BMT recipients have shown that ganciclovir is effective in the prevention of CMV disease compared with placebo, although there is no difference in survival between treatment groups.<sup>31,32</sup> In both studies, there was a significantly increased risk of neutropenia in patients treated with ganciclovir. However, it should be noted that these studies were performed before the widespread use of haematopoietic growth factors such as G-CSF. These can abrogate neutropenia when the two are used together but whether this would have clinical benefit remains to be proven.

Less information exists about foscarnet treatment of BMT patients. Several small uncontrolled studies have been performed (Table 8).

Study	Regimen	Result
Lönnqvist <i>et al</i> , 1993 <sup>33</sup>	Continuous infusion of foscarnet for 1 month compared with placebo	The study was stopped after two patients in the foscarnet group developed CMV pneumonia after the end of the foscarnet treatment period
Reusser <i>et al</i> , 1992 <sup>34</sup>	Foscarnet 120 mg/kg for 1 month followed by 60 mg/kg until 12 weeks after transplant	None of the 12 patients in this study developed CMV disease
Bacigalupo <i>et al</i> , 1994 <sup>35</sup>	Foscarnet 60 mg three times daily for 5 days followed by 90 mg/kg 3 days per week for 3 months	There was a reduction of antigenaemia frequency in this study compared with historical controls and one patient developed CMV disease.

Table 8: Summary of studies of foscarnet prophylaxis<sup>33–35</sup>

## Prophylaxis in solid organ transplant recipients

### Low potency prophylaxis

Aciclovir prophylaxis can reduce CMV disease in solid organ transplant recipients. In renal transplant patients, Balfour *et al* (1989) compared aciclovir prophylaxis (800 mg–3200 mg/day) with placebo in a randomized trial.<sup>36</sup> There was a significant reduction in the incidence of CMV disease from 29% in the placebo group to 7.5% in the aciclovir arm (Figure 4), but there was no difference in survival between the two groups.

Preliminary results from a randomized trial of prophylaxis with valaciclovir versus placebo in low-risk renal transplant recipients show that the incidence of CMV disease was reduced from 5% in the placebo arm to 1% in those receiving valaciclovir ( $P < 0.05$ ).<sup>37</sup>

Patients were randomized 1:1 to receive either valaciclovir (2000 mg four times per day) or

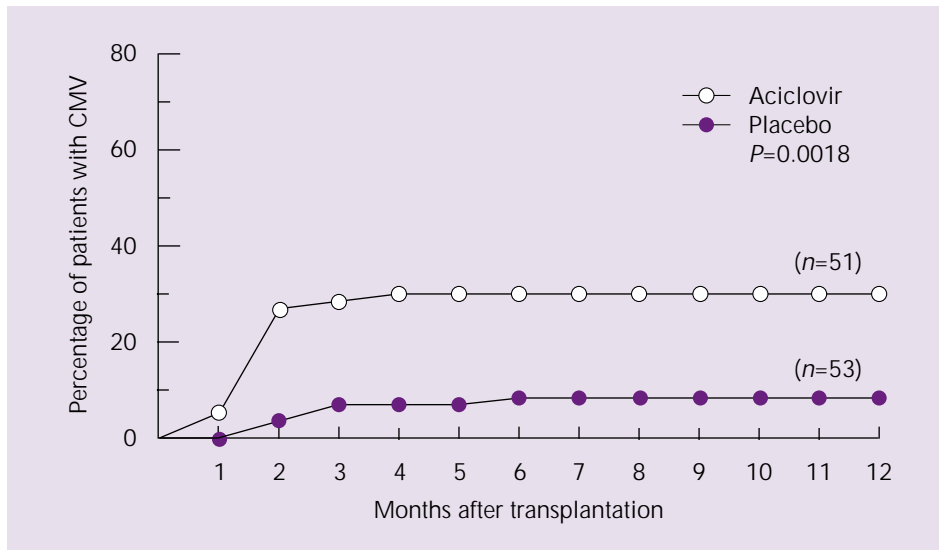


Figure 4: Percentage of renal transplant recipients with CMV disease during prophylaxis with aciclovir or placebo<sup>36</sup>

matching placebo for 90 days post-transplant. The study was conducted in two parts to compare patients who were CMV seropositive prior to transplant with patients who were CMV seronegative.

The five-fold decrease in the incidence of CMV disease was observed in CMV seropositive transplant recipients. At 90 days post-transplant, 11 of the 204 seropositive patients on placebo progressed to CMV disease whereas no cases were observed in the 204 seropositive patients receiving valaciclovir. Analysis of the full survival curve to 1 year showed that two cases of CMV disease were reported after 90 days in the valaciclovir arm.

Further analyses of this study are underway and results for seronegative transplant recipients are expected towards the end of 1997.

In liver transplant recipients, aciclovir treatment reduced the incidence of CMV infection and the frequency of CMV disease in a randomized, open study, but had no effect on survival.

### High potency prophylaxis

Positive results on the prevention of CMV disease have been achieved with prophylaxis with high potency drugs, although studies are often difficult to interpret due to the small numbers of patients involved. Merigan *et al* (1992) showed that in CMV-seropositive heart transplant recipients, ganciclovir treatment led to a significant reduction in CMV disease.<sup>38</sup> In seronegative patients with seropositive donors, ganciclovir surprisingly did not show any difference compared with placebo in CMV disease.

In seronegative renal transplant recipients who received grafts from seropositive donors, ganciclovir was compared with no therapy. Although there was a 30% difference in the frequency of CMV disease, significance was not achieved due to the small numbers of patients in the trial. A study in a similar group of patients compared ganciclovir with immunoglobulin.<sup>39</sup> When these patients were compared with an historical control group receiving no prophylaxis, there was no difference between ganciclovir and standard immunoglobulin on the development of CMV disease, but

both treatment groups had a significantly lower incidence of CMV disease than the control group.

Two studies have compared ganciclovir with aciclovir in liver transplant recipients. Winston *et al* (1995) showed that ganciclovir (6 mg/kg/day) was more effective in preventing CMV disease and infection than aciclovir (Figure 5), with a comparable side-effect profile and no difference in survival.<sup>40</sup> Martin *et al* (1994) compared 14 days of ganciclovir plus aciclovir therapy with aciclovir therapy alone.<sup>41</sup> The frequency of CMV disease was similar compared with the Winston study.<sup>40</sup> Again there was a strong reduction in the risk of CMV disease in the ganciclovir plus aciclovir group.

In lung transplant patients receiving ganciclovir for 12 weeks, or ganciclovir for 3 weeks, then aciclovir for 9 weeks,<sup>42</sup> there was a substantial reduction in CMV pneumonia and virus shedding in the ganciclovir 12 week group, and a significant reduction in pneumonitis at 1 year post-transplant. Two years after the transplant, however, the positive benefits of ganciclovir had disappeared and there were no differences between the groups.

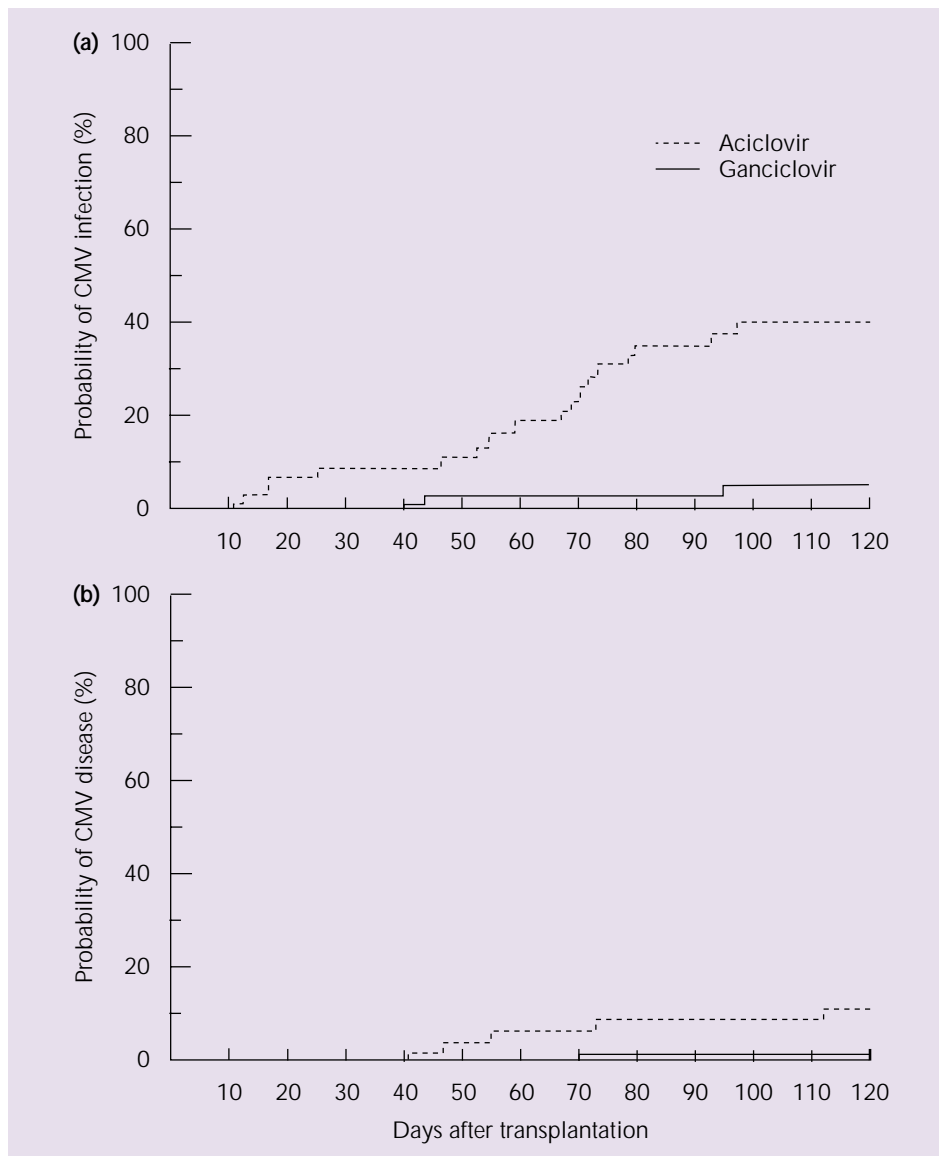


Figure 5: Kaplan-Meier product limit estimates of the probability of CMV infection (a) or disease (b) after liver transplantation in patients treated with aciclovir or ganciclovir<sup>40</sup>

## Pre-Emptive Therapy

Pre-emptive treatment strategies can be employed when CMV infection is detected before symptoms of CMV disease have developed. The benefits of pre-emptive therapy include:

- Treatment is initiated when virus load is low
- Effective at preventing CMV disease
- Selection of patients for this strategy will reduce the risk of treatment-related side-effects because fewer patients are exposed to drug
- As therapy is delayed until CMV is first detected, this may allow the development of an immune response

However, pre-emptive therapy strategies demand intensive surveillance and there is a risk that CMV disease will develop before diagnostic tests become positive.

### *Pre-emptive therapy in BMT recipients*

Goodrich *et al* (1993) evaluated a pre-emptive strategy in a trial with ganciclovir and placebo.<sup>31</sup> Ganciclovir therapy (5 mg/kg twice daily for 5 days, followed by once daily until Day 100 post-transplant) was initiated following a positive shell vial culture from blood, urine or throat. Ganciclovir therapy reduced the incidence of CMV disease at Day 100 from 29% in the placebo arm to no patients in the ganciclovir arm. Some patients developed late CMV disease and died in the ganciclovir arm after therapy was stopped. However, there was a survival advantage for the ganciclovir arm both at the time that ganciclovir prophylaxis was stopped and at follow-up 3 months after the end of prophylaxis.

Schmidt *et al* (1991) also tested a pre-emptive strategy in 104 BMT patients.<sup>43</sup> Patients who had positive CMV cultures from bronchoalveolar lavage (BAL) were randomized to receive either ganciclovir (5 mg/kg twice daily for 14 days then five times per week until Day 120) or no therapy. Ganciclovir significantly reduced the incidence of CMV pneumonia and death; 70% of patients receiving no therapy developed CMV pneumonia or died, compared with only 5% of patients receiving ganciclovir. A third group in this study were patients who were negative for CMV on BAL; these patients had intermediate outcomes.

These studies suggest that a pre-emptive therapy strategy is an improvement over no treatment. A study by Boeckh *et al* (1995) compared a pre-emptive ganciclovir strategy based on antigenaemia with prophylaxis with ganciclovir.<sup>44</sup> Up to Day 100, there was lower efficacy with pre-emptive therapy than with ganciclovir prophylaxis for prevention of CMV disease. However, the increased risk of late CMV disease meant that there was no difference in CMV disease between the two groups at Day 180. There was also no difference in survival.

There are currently no controlled trials comparing ganciclovir with foscarnet in BMT patients. Bacigalupo *et al* (1994) looked at T-cell depleted BMT patients.<sup>45</sup> Those patients experiencing problems with marrow function were given foscarnet; other patients were given ganciclovir. In this small study, there was no clear difference between the two treatment groups in the risk for development of CMV disease.

Ljungman *et al* (1996) have performed a preliminary investigation in BMT patients using a low dose of intravenous foscarnet 60 mg/kg twice daily as pre-emptive therapy

based on leucocyte PCR.<sup>46</sup> No patients developed CMV disease and there was no renal toxicity at this dose. These two studies have provided the basis for an ongoing randomized trial of ganciclovir versus foscarnet.

Finally, the combination of ganciclovir and foscarnet has been tested by Bacigalupo *et al* (1995) in a high-risk group (high level antigenaemia, T-cell depleted patients).<sup>47</sup> Of 15 patients, only one developed CMV disease. Importantly, no increased toxicity was observed. These findings support results from the Study of Ocular Complications in AIDS (SOCA) trial.

### *The use of diagnostic tests in deciding when to implement pre-emptive therapy*

In a study in BMT patients, Einsele *et al* (1995) randomized patients to ganciclovir therapy based on detection of CMV by either PCR analysis or shell vial assay.<sup>48</sup> The use of the more sensitive PCR test allowed therapy to be initiated earlier and led to a reduction of the risk for CMV disease and for CMV-associated deaths and the risk for side-effects. In another study, Ljungman *et al*<sup>49</sup> compared patients diagnosed by PCR with matched historical controls and obtained similar results. In addition, this study also analysed the levels of CMV DNA in a semi-quantitative fashion and found that patients with higher levels of CMV DNA were more likely to develop CMV disease.

### *Pre-emptive therapy in solid organ transplant recipients*

In liver transplant recipients, Cohen *et al* (1993) investigated the use of ganciclovir for pre-emptive therapy versus prophylaxis.<sup>50</sup> No differences in either CMV infection or survival were seen between the two strategies. Pre-emptive ganciclovir therapy for 7 days has also been compared with aciclovir prophylaxis in this population. Only 4% of patients receiving short pre-emptive ganciclovir therapy developed CMV disease, compared with 29% of patients in the aciclovir prophylaxis group.<sup>51</sup>

## The Impact of Different Strategies on Disease Outcome in Cytomegalovirus

Based on the results outlined above, the probabilities of achieving various outcomes with each treatment strategy can be compared. If there is a standard risk for disease and it is assumed that pre-emptive and prophylactic strategies are equally effective for disease prevention, several outcomes are apparent:

- ◆ There is a risk of breakthrough disease
- ◆ Some patients will receive unnecessary antiviral treatment
- ◆ Some patients will receive unnecessary prophylaxis
- ◆ Some disease will be prevented

## Resistance

### *CMV resistance to antiviral drugs in the transplant population*

Resistance of CMV to antiviral drugs has been implicated in drug failure for the last 10 years. The development of resistant viral strains has allowed the molecular mechanisms of action of antiviral agents to be investigated. For example, ganciclovir-resistant CMV strains were used to elucidate the anti-CMV activity of aciclovir.

Virus resistance to a drug develops over time. Treatment of transplant recipients is usually for short periods, and therefore resistance is less likely to develop.

Clinical data in transplant populations are few. In a survey of 28 centres performing BMT, 19 reported CMV resistance. However, of 25 patients with suspected CMV resistance, laboratory tests proved resistance in only two. For the remainder of the patients, CMV resistance was suspected only on clinical grounds.

Slavin *et al* (1993) looked at ganciclovir resistance in 12 BMT patients with CMV pneumonia.<sup>52</sup> Virus isolated from BALs showed that after 9 days of therapy only one patient had a resistant virus strain. There was also no clear association between the occurrence of resistance and clinical outcome.

### *Treatment of resistance*

There is little information about the treatment of solid organ transplant recipients who have CMV infection which is resistant to drugs. In the BMT setting, one patient who developed CMV pneumonia and viraemia from a ganciclovir-resistant virus strain was given foscarnet and the disease was treated. When the same patient had a relapse, isolates were found to be resistant to both ganciclovir and foscarnet.

## Summary and Management Recommendations

### *Recommended strategies for the treatment, prophylaxis and pre-emptive therapy of CMV*

The response to treatment of established disease caused by CMV is not optimal under certain circumstances. Physicians should consider the development of CMV disease as a failure of pre-emptive therapy and use the strategies below to reduce CMV disease.

#### *Established disease*

##### *Solid organ transplant recipients*

In solid organ transplant recipients, CMV pneumonitis is treated for 2–4 weeks with ganciclovir (plus immunoglobulin in some centres). Other CMV disease in these patients is treated with ganciclovir for 2 weeks.

##### *Category 2 Recommendation*

It is unclear whether addition of immunoglobulins to existing treatment regimens has a benefit to solid organ transplant recipients (see Chapter 3).

##### *Category 2 Recommendation*

Some experts would add immunoglobulin to the treatment regimen in severely ill solid organ transplant recipients.

##### *Category 3 Recommendation*

Combination therapy with antivirals may be more effective for the treatment of established CMV disease. Combinations of ganciclovir and foscarnet, new drugs like cidofovir, new types of immunoglobulin preparations (e.g. monoclonal antibodies), duration of treatment and resistance should be investigated.

BMT recipients with CMV pneumonitis diagnosed by BAL should be treated with ganciclovir plus CMV immunoglobulin.

##### *Category 2 Recommendation*

There is no recommendation on which type of immunoglobulin (polyspecific versus hyperimmune) should be used.

Future studies of treatment of established disease should address issues including:

- ◆ The use of combination therapy (e.g. ganciclovir with foscarnet) for established CMV disease
- ◆ The use of new drugs (e.g. cidofovir), and their effects on viraemia
- ◆ The use of new types of immunoglobulin preparations (e.g. monoclonal antibodies)
- ◆ The duration of treatment
- ◆ The addition of immunoglobulins to therapy
- ◆ The problem of resistance

### *Prophylaxis and pre-emptive therapy*

#### *BMT recipients*

BMT recipients should be considered in three groups according to risk (see page 34).

In high-risk patients, monitoring for CMV infection using antigenaemia or PCR is




# Chapter 2

advised. The two therapy options for these patients are high potency prophylaxis or pre-emptive therapy with or without low potency prophylaxis.

### Category 1 Recommendation

For intermediate risk patients, ganciclovir prophylaxis is not recommended due to cost and the risk of side-effects. Pre-emptive therapy with ganciclovir with or without low potency prophylaxis is suggested.




### Category 1 Recommendation

BMT Risk Categories	
 HIGH	Allogeneic HLA mis-match or unrelated donor, all serogroup combinations except D-/R-, acute GVHD, T-cell depletion
 INTERMEDIATE	Allogeneic HLA matched, all serogroup combinations except D-/R-
 LOW	Allogeneic HLA matched, D-/R- Autologous, all serogroup combinations

HLA, histocompatibility leucocyte antigen; GVHD, graft versus host disease; D, donor; R, recipient.

For low-risk patients, filtered blood products are recommended. There is no need for any antiviral prophylaxis.

### Category 1 Recommendation

BMT Prophylaxis/Pre-Emptive Therapy		
Risk	Regimen	Recommendation Category
 HIGH	Iv GCV prophylaxis	1
	Iv GCV pre-emptive therapy +/- low potency prophylaxis	1
 INTERMEDIATE	Iv GCV pre-emptive therapy +/- low potency prophylaxis	1
 LOW	CMV-/filtered blood	1

iv GCV, intravenous ganciclovir.

The pre-emptive therapy strategy currently in widespread use is to initiate ganciclovir therapy once CMV is detected in blood or BAL. Initially a 2-week course is given with continuous monitoring for virus. A maintenance dose is continued until tests become negative. G-CSF is often used for treatment of ganciclovir-associated neutropenia (*Category 3 Recommendation*); to avoid neutropenia an alternative strategy in patients with normal renal function would be use to foscarnet.

### Category 2 Recommendation

In non-responding patients, the combination therapy of ganciclovir plus foscarnet should be considered. However, this needs to be tested in a prospective trial.

### Category 3 Recommendation

### Solid Organ Transplant Risk Categories (Risk Factors)

HIGH	D <sup>+</sup> /R <sup>-</sup> Use of ALG in all serogroups except D <sup>-</sup> /R <sup>-</sup>
INTERMEDIATE	D <sup>+</sup> /R <sup>+</sup> , D <sup>-</sup> /R <sup>+</sup>
LOW	D <sup>-</sup> /R <sup>-</sup>

ALG, antilymphocyte globulin.

CMV-seronegative patients who are candidates for BMT should be maintained as seronegative by use of screened or filtered blood products. This will reduce the risk of the recipient developing a primary infection.

*Category 3 Recommendation*

### Solid organ transplant recipients

Solid organ transplant recipients should be considered in three groups according to risk factors and transplant category.

### Solid Organ Transplant Risk Categories (Transplant Category)

HIGH	Lung, bowel
INTERMEDIATE	Heart, liver, pancreas
LOW	Kidney

According to these categories, prophylaxis and pre-emptive therapy strategies can be recommended as follows:

### Low-Risk Solid Organ (Kidney) Transplant Prophylaxis/Pre-Emptive Therapy

Risk factors	Regimen	Recommendation Category
HIGH	GCV>ACV. CMV Ig	2
	Pre-emptive therapy	3
	GCV + ALG	1
INTERMEDIATE	ACV	2
	No prophylaxis	
LOW	CMV-/filtered blood	3

GCV, ganciclovir; ACV, aciclovir; CMV Ig, CMV immunoglobulin.

### Intermediate Solid Organ (Heart, Liver, Pancreas) Transplant Prophylaxis/Pre-Emptive Therapy

Risk factors	Regimen	Recommendation Category
HIGH	GCV (3 months)	1
	Pre-emptive GCV	2
	GCV + ALG	2
INTERMEDIATE	GCV (1 month–3 months)	1
	Pre-emptive GCV	2
	ACV prolonged	3
LOW	CMV-/filtered blood	3

## High Risk Solid Organ (Lung, Bowel) Transplant Prophylaxis/Pre-Emptive Therapy

Risk factors	Regimen	Recommendation Category
HIGH	GCV (3 months) + CMV Ig +/- pre-emptive therapy	3
INTERMEDIATE	GCV (1–3 months) +/- pre-emptive therapy	3
LOW	CMV -/filtered blood	3

In some studies performed in liver transplant recipients, results are similar for prophylaxis and pre-emptive therapy; therefore, further studies are necessary to thoroughly compare the two strategies in terms of efficacy, safety and cost.

Future studies of prophylaxis and pre-emptive therapy should address issues including:

- The use of combination therapy (e.g. ganciclovir with foscarnet) for prophylaxis and pre-emptive therapy in a high-risk group of patients (according to risk and type of transplant)
- CMV diagnostic markers
- Different types of immunosuppression and new agents
- Duration of maintenance pre-emptive therapy
- Cost-effectiveness of different regimens

It is recommended that new antiviral drugs should always be evaluated in controlled clinical trials, although it is recognized that the numbers of patients may be limiting. To overcome this limitation, future clinical trials should be multinational to allow small numbers of patients in different countries to be analysed together, and ganciclovir controlled.

### Resistance

The incidence of resistance in transplant patients is low because drug therapy is usually for short periods of time and current drugs are not very effective. CMV resistance is usually confined to HIV-infected individuals who take therapy over long time periods while they are profoundly immunocompromised. More information on resistance in the HIV-infected population can be found in reference 53.

As a result, there are few data on resistance of CMV to antiviral treatments in the transplant population.

Disease progression may not be related to resistance. Poor efficacy and poor drug delivery are often the underlying reasons for lack of response to treatment rather than resistance.

Susceptibility testing is very difficult; there is large variation in the reliability of tests between different laboratories and the process takes a long time. *In vivo* susceptibility testing using an antigen-type or immunofluorescence test or molecular technique should be evaluated.

#### Research Need

It is recommended that transplant patients should only be tested for resistance if it is clinically suspected; continual monitoring is not cost-effective.

It is recommended that rapid assays of viral load are developed for use in transplant patients to monitor which patients fail to respond promptly to treatment.

## New Approaches in the Management of Cytomegalovirus

Although treatment, prophylaxis and pre-emptive therapy strategies can be effective against cytomegalovirus (CMV) infection and disease, new approaches may provide even greater benefits for some patients.

This chapter reviews developments in vaccines to prevent CMV infection, and immunotherapeutic approaches, both with immunoglobulins and T-cell adoptive immunotherapy.

### Immunity and Cytomegalovirus Disease – The Rationale for Immunization

There is substantial evidence from a number of sources that immunity to CMV, induced by naturally acquired infection and, in some cases, by active and passive immunization, can provide protection from disease. For example, in most organ transplant settings CMV disease is more common and more severe in patients who develop primary infection after transplantation than in those who have pre-transplant immunity from previous CMV infection. Studies of both active and passive immunization have shown at least modest benefit in solid organ transplant patients.

The recognition two decades ago that mothers who had CMV infection more than a year prior to conception could still transmit CMV transplacentally, led to pessimism regarding the feasibility of using active immunization to prevent congenital infection. However, recent studies have shown that preconception maternal immunity lessens the severity of disease in congenital infection (Table 1).<sup>1</sup>

Sequelae	Primary (%)	Recurrent (%)	P
SNHL	18/120 (15)	3/56 (5)	0.05
Bilateral SNHL	10/120 (8)	0/56 (0)	0.02
Speech detection best ear 60 dB	9/120 (8)	0/56 (0)	0.03
IQ <70	9/68 (13)	0/32 (0)	0.03
Retinitis	7/112 (6)	1/54 (2)	0.20
Neurologic abnormalities	8/125 (6)	1/64 (2)	0.13
Death	3/125 (2)	0/64 (0)	0.29
Any sequelae	31/125 (25)	5/64 (8)	0.003

SNHL, sensorineural hearing loss.

Table 1: Sequelae in children with congenital CMV infection according to type of maternal infection<sup>1</sup>

Central nervous system (CNS) sequelae were seen in 25% of babies with congenital CMV infection born to mothers who had primary infection during pregnancy, compared with 8% of infected babies whose mothers had been infected well before pregnancy. Differences in disease severity were even more notable; mental retardation and bilateral hearing loss were seen only in babies born after primary maternal infection.

There is also evidence that immunity from prior maternal infection reduces the rate of congenital CMV infection. Follow-up of women from one pregnancy to the subsequent one revealed that those who were initially seropositive had an approximately five-fold reduced risk of congenital CMV infection in the subsequent pregnancy compared with women who were initially seronegative.<sup>2</sup>

Studies in experimental animals have provided evidence that immunity to the CMV envelope glycoprotein gB, the major target of neutralizing antibody, can protect against disease. In a murine model, both active and passive immunization to gB protected mice from a lethal challenge with murine CMV.<sup>3</sup> A study in pregnant guinea pigs showed that immunization with a guinea pig CMV envelope glycoprotein equivalent to gB reduced the rate of transplacental transmission and CMV disease in infected pups.<sup>4</sup> Improved understanding of the role of various CMV proteins is likely to lead to new recombinant vaccines. Selected CMV proteins of particular interest are listed in Table 2. Although there are no licensed CMV vaccines at the present time and the role of passive immunization in immunocompromised patients remains controversial, new developments in immunotherapy are on the horizon.

Protein	Description
gB	Envelope glycoprotein, major target of neutralizing antibody
gH	Envelope glycoprotein, target of neutralizing antibody
pp65	Abundant tegument protein
pp28	Tegument protein
pp150	Tegument protein
pp82	Nonstructural protein, IgM antibodies
pp72, pp89	Immediate early proteins

Table 2: Immunogenic human CMV proteins

## Active Immunization – Progress with Cytomegalovirus Vaccines

Vaccines against CMV infection are under development. Although no vaccine is currently available, several preparations are undergoing preclinical evaluation and clinical trials with both Towne strain live CMV vaccine and a new recombinant glycoprotein B vaccine have yielded valuable new information on human responses to active CMV immunization.

### *Clinical trials of active immunization*

The first CMV vaccine tested in humans was a live virus vaccine made from the AD169 laboratory adapted strain.<sup>5</sup> This vaccine was given to healthy adult volunteers; 25 of 26 who received 10 000 TCID<sub>50</sub> subcutaneously seroconverted. Local reactions were common but CMV was not isolated from any of the vaccinees. This vaccine does not appear to have been investigated beyond initial Phase I studies. The Towne strain of CMV was attenuated by multiple *in vitro* passage and used in an investigational live virus vaccine.<sup>6</sup> Healthy subjects developed antibody and lymphocyte proliferative

responses after vaccination with Towne vaccine. A small number of volunteers who received Towne vaccine were challenged by a low passage wild type CMV (Toledo); only one out of seven Towne recipients had an illness following challenge, compared with all four seronegative volunteers.<sup>7</sup> These initial encouraging results were followed by efficacy trials.

### Vaccination of healthy adults

A recent trial of Towne vaccine for prevention of CMV infection in healthy adults exposed to CMV shedding pre-school age children found no difference in rate of infection between vaccine and placebo recipients, although there was a correlation between high levels of neutralizing antibody and protection from subsequent CMV infection.<sup>8</sup>

### Vaccination in renal transplant recipients

Plotkin *et al* (1991) looked at the effect of Towne strain vaccine in renal transplant recipients at high risk for post-transplant CMV infection and disease.<sup>9</sup> Patients who had no antibody to CMV and who received kidneys from seropositive donors were randomized to receive Towne vaccine or placebo pre-transplant. There was no statistically significant difference in the rate of CMV infection or disease between vaccine and placebo recipients. However, there was a decrease in the rate of CMV disease classified as severe based on a scoring system (Table 3).

	Vaccine (%) (n=36)	Placebo (%) (n=31)	P
● Infection	32 (89)	24 (77)	>0.2
● Disease	14 (40)	17 (55)	0.2
● Median score	0	5	0.03
● Score 10	2 (5.6)	8 (25)	0.09

Table 3: Efficacy of Towne strain live-attenuated CMV vaccine in seronegative recipients of kidneys from seropositive donors<sup>9</sup>

Although prevention of severe CMV disease among renal transplant patients with primary CMV infection is clearly worthwhile, the potential target population (seronegative recipients of kidneys from seropositive donors) is small, making the development and manufacture of a vaccine for this limited purpose unattractive. In addition, other approaches to prevention of CMV disease in solid organ transplant recipients, including antiviral prophylaxis or pre-emptive therapy and passive immunotherapy, have shown great promise.

The future of Towne strain live CMV vaccine is limited. Although no evidence of reactivation of vaccine virus was found even in immunocompromised patients, concerns remain regarding the potential long-term consequences of immunizing humans with live CMV. Standardization of vaccine lots for immunogenicity could well be a problem for a CMV vaccine, as it was for varicella zoster virus (VZV). Better understanding of the molecular basis for virulence, *in vivo* replication and attenuation is needed.

### Future issues for vaccine development

The two major management goals for CMV vaccines are:

- Prevention of CNS damage from congenital CMV infection
- Prevention of CMV disease in immunocompromised patients

Achieving these goals will be facilitated by a better understanding of how the components of the immune response to specific CMV proteins protect from infection and disease.

At least two new CMV vaccines have undergone development and evaluation. A new recombinant CMV vaccine was in Phase I and II trials in the USA. The Chiron Biocine CMV gB vaccine was based on the envelope glycoprotein gB, the major target of neutralizing antibody. The recombinant molecule was mutagenized to facilitate *in vitro* production of the glycosylated protein by Chinese hamster ovary cell culture. The vaccine was formulated with a new proprietary adjuvant. Although preliminary results from human Phase I trials in which vaccinees received three doses on a 0, 1 and 6 month schedule revealed that the vaccine was safe and immunogenic, this vaccine will not be developed further. Recipients developed antibodies to gB and neutralizing antibodies as well as brisk lymphocyte proliferative responses.<sup>10</sup> The duration of the vaccine-induced immunity and whether the CMV gB vaccine can protect from infection and disease will be defined by further studies. A replicating recombinant CMV gB vaccine using a canarypox vector has also been developed and looks promising in preclinical trials.<sup>11</sup>

The observation that immunity from naturally acquired CMV infection is unable to prevent re-infection has led to pessimism. However, it is clear that immunity to CMV induced by past infection decreases the rate and severity of disease, and it is likely that vaccine induced immunity will eventually be able to do the same.

## Passive Immunization with Cytomegalovirus Immunoglobulins

### *The role of antibodies in the control of CMV infection*

Although it is clear from clinical and experimental experience that impaired cell-mediated immunity leads to greater frequency and severity of CMV disease, the role of the humoral immune system cannot be overlooked. Virus-specific antibody can perform a number of important functions in host defence, many of which operate even when cell-mediated immunity is impaired. Table 4 lists the mechanisms through

Inactivate or decrease the infectivity of virus:

- ◆ aggregation of virus
- ◆ altering virus surface molecules necessary for cell attachment
- ◆ neutralization of virus

Facilitate clearance of virus:

- ◆ activation of complement and/or opsonophagocytosis
- ◆ Fc-receptor mediated phagocytosis

Lysis of virus-infected cells:

- ◆ antibody-dependent complement-mediated pathways
- ◆ antibody-dependent cellular toxicity

Table 4: Influence of antibodies on controlling viral infection

which antibody facilitates control and clearance of viral infections in general. Animal models of CMV infection have shown that passive immunization with antibody to the envelope glycoprotein gB can prevent lethal infection.<sup>12</sup> The ability of antibody alone

to function in control of virus infection suggests a role for passive immunization for prevention or treatment of CMV disease, and there is evidence from clinical trials that this approach can be beneficial.

CMV immunoglobulin treatment has been shown to have a beneficial effect in high-risk solid organ transplant recipients. While there are many published trials on the use of immunoglobulins in these patients (Table 5), properly controlled trials are few.<sup>13-20</sup>

Trial	Subjects	Design	Results
Stratta <i>et al</i> , 1992 <sup>13</sup>	Liver transplant, OKT3-treated (n=100)	Randomized Immunoglobulin vs aciclovir	No change in disease rate in either group
Conti <i>et al</i> , 1993 <sup>14</sup>	Renal transplant, D <sup>+</sup> /R <sup>-</sup> (n=71)	Randomized Immunoglobulin vs no treatment	Severe CMV disease reduced in the treatment group. No change in overall CMV syndromes
Nicol <i>et al</i> , 1993 <sup>15</sup>	Renal transplant, D <sup>+</sup> /R <sup>-</sup> (n=361)	Uncontrolled Hyperimmune globulin vs aciclovir	Less CMV disease than expected from experience
Bailey <i>et al</i> , 1993 <sup>16</sup>	Solid organ transplant, D <sup>+</sup> /R <sup>-</sup> (n=21)	Randomized Ivlg vs ganciclovir	High rates of infection and disease No difference by regimen
Conti <i>et al</i> , 1994 <sup>17</sup>	Renal transplants, D <sup>+</sup> /R <sup>-</sup> (n=51)	Randomized Ivlg vs ganciclovir	No differences by regimen Both effective compared with historical controls
Dunn <i>et al</i> , 1994 <sup>18</sup>	Solid organ transplant (n=266)	Aciclovir vs ganciclovir + immunoglobulin	Aciclovir recipients had less CMV disease
Snydman <i>et al</i> , 1987 <sup>19</sup>	Renal transplants (n=59)	Randomized Ivlg vs no treatment	Incidence of CMV disease reduced from 60% in controls to 21% in Ivlg-treated patients
Snydman <i>et al</i> , 1993 <sup>20</sup>	Liver transplant (n=141)	Randomized, double-blind. Immunoglobulin vs placebo	Immunoglobulin treatment decreased the incidence of CMV disease from 26% in the placebo group to 12%. There was no effect in D <sup>+</sup> /R <sup>-</sup> patients

Table 5: Trials of CMV immunoglobulin or intravenous immunoglobulin (Ivlg) in solid organ transplant patients<sup>13-20</sup>. D, donor; R, recipient

In one of the first controlled studies of passive immunization to prevent CMV disease in renal transplant recipients, clinical and virological outcomes were compared between patients receiving CMV immunoglobulin and a control group receiving albumin.<sup>19</sup> The results showed modest but statistically significant reductions in the incidence of CMV syndrome and fungal infections in the group receiving CMV immunoglobulin compared with the control group (Table 6). This study formed the foundation for the use of CMV immunoglobulin in solid organ transplant patients to prevent CMV disease. However, the effects were modest and some patients still developed CMV disease.

Similar trends have been observed in another placebo-controlled trial in liver transplant recipients who received CMV immunoglobulin. There were significant

Outcome	Controls (%) (n=35)	CMV immunoglobulin group (%) (n=24)	P
CMV syndrome	21 (60)	5 (21)	<0.01
Fungal infection	7 (20)	0 (0)	0.05
Death	5 (14)	1 (4)	
Graft loss	10 (28)	4 (17)	
Viraemia	15 (43)	6 (25)	
Virus excretion	20 (57)	13 (54)	
Seroconversion	27 (77)	17 (71)	

Table 6: Efficacy of intravenous CMV immunoglobulin – results from a randomized trial in D<sup>+</sup>R<sup>-</sup> renal transplant recipients<sup>19</sup>

reductions in fungal disease and in severe disease, and positive trends in death and overall CMV disease in the immunoglobulin group.<sup>20</sup>

The renal transplant study described above was performed in patients who were donor positive/recipient negative (D<sup>+</sup>R<sup>-</sup>) only, while the study in liver transplant recipients included all donor/recipient combinations. When the data from the liver transplant study were analysed by serostatus, there was no difference in the rate of infection or the rate of disease in the D<sup>+</sup>R<sup>-</sup> group between treated and placebo groups. When all seropositive recipients were analysed together, there was a statistically significant reduction in the rate of infection and a reduction in the rate of disease in the immunoglobulin-treated group (Table 7). It is unclear why a reduction should occur in the group which might be considered least likely to benefit, while there was no benefit in the group (D<sup>+</sup>R<sup>-</sup>) which was expected to have benefited most.

Group	Disease*		Severe disease <sup>†</sup>	
	CMV immunoglobulin	Placebo	CMV immunoglobulin	Placebo
D <sup>-</sup> R <sup>-</sup>	0/19	4/25	0/19	2/25
D <sup>-</sup> R <sup>+</sup>	2/18	5/16	0/18	4/16
D <sup>+</sup> R <sup>+</sup>	1/13	3/12	1/13	4/12
D <sup>+</sup> R <sup>-</sup>	10/19	10/19	7/19	9/19

\*P=0.08; <sup>†</sup>P<0.01.

Table 7: A controlled trial of CMV immunoglobulin in liver transplant patients: stratified analysis by D/R serostatus<sup>20</sup>

The prevention of CMV disease by use of CMV immunoglobulin has some beneficial effects in D<sup>+</sup>R<sup>-</sup> renal transplant patients. However, in other transplant settings, it is difficult to ascribe a role for CMV immunoglobulin in the prevention of CMV disease, based on controlled studies.

In bone marrow transplant (BMT) recipients, neither CMV immunoglobulin nor intravenous immunoglobulin preparations should be used alone to prevent CMV infection. However, intravenous immunoglobulin decreases the incidence of graft versus host disease (GVHD), and in combination with antiviral therapy both CMV

immunoglobulin and intravenous immunoglobulin are effective in the treatment of CMV pneumonia. There is probably no role for CMV immunoglobulin in BMT recipients as a preventative agent, unless used in combination with antiviral therapy.

When making recommendations on the use of immunoglobulin therapy, the cost and benefit of antiviral prophylaxis needs to be considered. If CMV immunoglobulin is used alone as prophylaxis in solid organ transplant recipients, virologic screening that would allow pre-emptive antiviral therapy to be initiated should also be employed.

## Adoptive Immunotherapy

Apart from the development of vaccines, other approaches to the future management of CMV infections are under investigation. Adoptive immunotherapy is a novel technique in which CMV-specific T-cells are used to reconstitute an immunodeficient host's cellular immune response to CMV.<sup>21,22</sup> The initial studies have been performed in allogeneic BMT recipients using CD8 CMV-specific cytolytic T-lymphocyte (CTL) clones isolated from the respective bone marrow donor, cultured *in vitro* and then infused into the recipient after the transplant. In preliminary clinical investigations the technique has been used successfully to reconstitute CMV-specific immunity and may hold promise as a future therapy for established CMV disease. This may be particularly relevant in the BMT setting because the increased use of prophylactic and pre-emptive antiviral drug therapy has resulted in an increase in the incidence of late-onset CMV disease, apparently as a consequence of a delay in the reconstitution of CMV-specific T-cell immunity by the suppression of CMV replication.

### *Rationale for adoptive immunotherapy*

The rationale for pursuing adoptive immunotherapy as a strategy for preventing CMV infection after BMT was initially provided by studies in BMT recipients in which the resolution of infection and protection from subsequent CMV disease correlated with the recovery of detectable CMV-specific T-cell responses in the peripheral blood,<sup>23-25</sup> and from animal model studies demonstrating that immunosuppressed mice could be protected from lethal murine CMV challenge by the infusion of CD8 murine CMV-specific T-cells.<sup>26</sup>

Fifty-six BMT recipients were prospectively evaluated in the first 100 days after transplant for recovery of MHC-restricted CD8 and CD4 CMV-specific T-cell responses using sensitive *in vitro* assays to determine if there was a correlation between endogenous reconstitution of CMV-specific T-cell immunity and protection from disease.<sup>25,27</sup> The assay for CD8 CTL involved culturing peripheral blood lymphocytes obtained from the patient at defined intervals after transplant with CMV-infected fibroblasts derived from a skin biopsy from either the histocompatibility leukocyte antigen (HLA)-matched donor or from the recipient. Cytotoxic responses of these cultured lymphocytes were then assessed against HLA matched and mismatched CMV-infected and uninfected target cells. The assay for CD4 CMV-specific T-helper cells involved culturing peripheral blood lymphocytes with a CMV antigen preparation derived from infected fibroblasts and assessing thymidine incorporation after 4 days. All immunocompetent CMV-seropositive individuals have detectable CD8 and CD4 CMV-specific T-cell responses with these assays, whereas CMV-seronegative individuals lack these responses.

Twenty-five of the 56 BMT patients recovered CD8 CTL responses and none of these individuals developed CMV pneumonia in the first 100 days, whereas the 31 patients who failed to develop a CTL response had a high incidence (50%) of CMV pneumonia. In this study, the reconstitution of T-helper cells also correlated with protection against disease, but two of seven patients with isolated recovery of CD4 CMV-specific T-helper cells but not CD8 CTL developed CMV pneumonia, suggesting that the recovery of the CD4 response alone was insufficient to provide protective immunity. The rationale for proceeding to an evaluation of T-cell adoptive immunotherapy for CMV disease prevention in BMT patients was therefore based on the following observations:

- ◆ CMV infection and disease are significant causes of morbidity and mortality after allogeneic BMT
- ◆ Post-transplant recovery of CD8 and CD4 CMV-specific T-cell responses correlates with protection from CMV disease
- ◆ CD8 and CD4 CMV-specific T-cell clones can be isolated from immunocompetent seropositive donors and propagated for use in adoptive immunotherapy of the immunodeficient recipient

### *Development of the adoptive immunotherapy technique*

The initial Phase I study of adoptive immunotherapy with donor T-cells for allogeneic BMT recipients was conducted using T-cell clones that were selected for reactivity with CMV-infected target cells; if unselected polyclonal donor T-cell populations had been infused, GVHD could occur and the additional immunosuppressive therapy required to treat GVHD could have negated any potential beneficial effects of the therapy.<sup>34</sup> The use of CMV-specific T-cell clones precluded any risk of GVHD and provided an opportunity to define the precise dose of CMV-specific T-cells necessary to reconstitute CMV immunity in immunodeficient hosts to levels present in immunocompetent hosts.

Animal model data and human studies suggested that CD8 CTL were the critical effector cells for controlling CMV, therefore the safety of infusing CD8 CMV-specific CTL clones were evaluated first. CTL exert their antiviral activity by several mechanisms. The recognition of virus infected cells by CD8 CTL occurs via engagement of the T-cell antigen receptor with class I MHC/peptide complexes expressed on the surface of the infected cell. This encounter leads to the T-cell releasing perforin which damages the target cell plasma membrane, and granzymes which enter the target cell and cause DNA fragmentation ensuring cell death (Figure 1).<sup>31,32</sup> CTLs also produce cytokines such as IFN $\gamma$  and TNF- $\alpha$  which exert antiviral activity on neighbouring uninfected cells.<sup>33</sup>

### *Identification of CMV target antigens*

One potential drawback with using individual T-cell clones in therapy was that only a limited repertoire of viral antigens could be targeted. CMV has a large genome encoding more than 200 open reading frames (ORFs). However, the immune response to many viruses is focused on a relatively small number of immunodominant target antigens. To search for potential immunodominant targets in CMV, metabolic

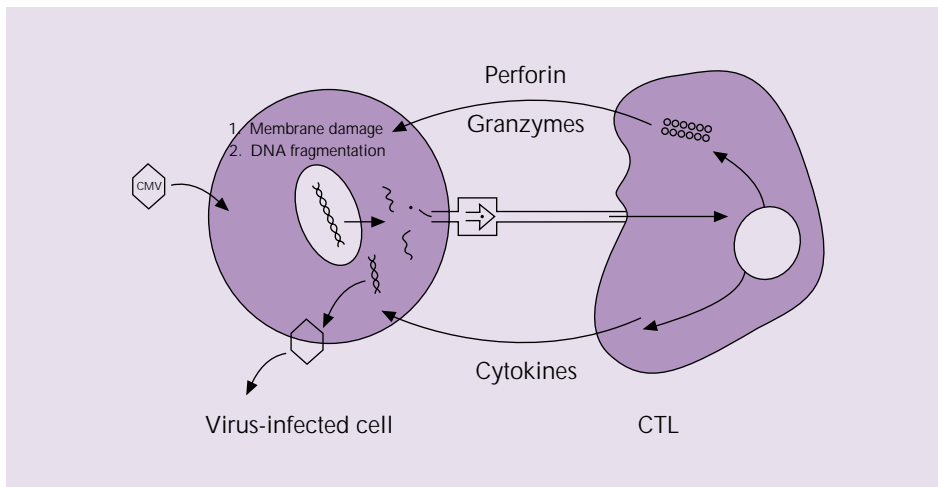


Figure 1: The mechanism of CTL-mediated antiviral effects.  
CTL, cytolytic T-lymphocyte

inhibitors were used to distinguish the contribution of two classes of CMV proteins as target antigens for CD8 CTL. These included:

- ◆ Structural proteins that enter the cell with the virion, such as matrix proteins
- ◆ Immediate early (IE), early (E) and late (L) proteins synthesized from the viral genome in the host cell after viral entry

The role of the viral structural proteins as targets for CD8 CTL was evaluated by infecting target cells with CMV in the presence of a transcription inhibitor (actinomycin D), which prevented the production of any IE, E or L proteins. The results of these experiments demonstrated that the CTL response in immunocompetent individuals is predominantly directed against structural proteins which enter the cell with the virion and that CTL to newly synthesized proteins comprise only a minor fraction of the host response.<sup>35</sup>

Using peptide digests and recombinant vaccinia vectors, matrix proteins such as pp65 and pp150 have subsequently been identified as the major antigens recognized by CMV-specific CTL in humans.<sup>36,37</sup> Recent studies have demonstrated that CMV encodes several genes in the US<sub>2</sub>-US<sub>11</sub> region which are expressed at IE or E times after infection and interfere with the stability and/or export of class I MHC molecules, thereby limiting the ability of the cell to present newly synthesized proteins to the immune system.<sup>38,40</sup> In addition, the CMV virion contains a protein that selectively precludes presentation of the abundant 72kD CMV major IE protein.<sup>41,42</sup> These results suggested that the selection of T-cell clones for use in adoptive therapy should be based on their ability to recognize structural virion proteins presented prior to the virus-induced inhibition of antigen presentation in infected cells.

### *Clinical experience with T-cell adoptive immunotherapy*

Walter *et al* (1995) have demonstrated that T-cell adoptive immunotherapy can successfully restore deficient immunity in BMT recipients.<sup>43</sup> A Phase I study of 14 BMT recipients evaluated the safety of infusing escalating doses of CD8 CTL and the ability of infused CTL to restore deficient immunity. Patients each received four infusions with the highest cell dose being  $1 \times 10^9$  cells/m<sup>2</sup> body surface area. No significant toxicities were observed and patients were able to receive the infusions as outpatients. In 11 of

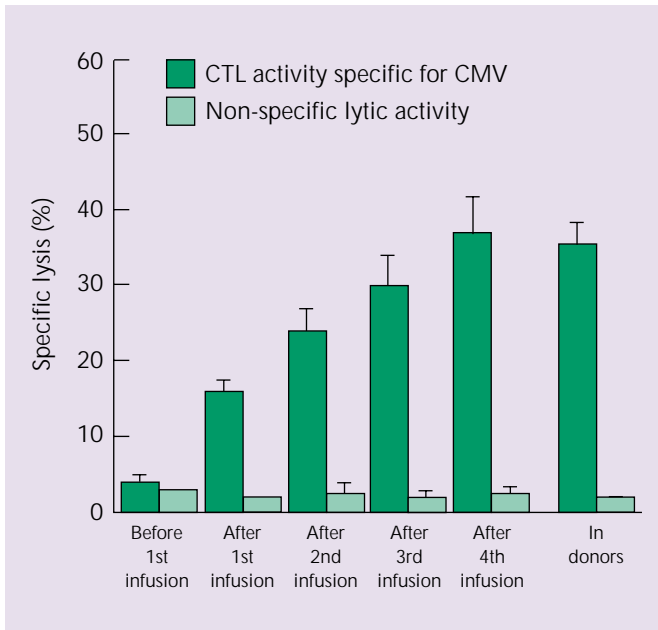


Figure 2: Cell-dose related reconstitution of CMV-specific CD8<sup>+</sup> CTL responses in 11 BMT recipients<sup>43</sup>

the 14 patients, there was no detectable CTL response to CMV before therapy was initiated on Day 28 to 42 after BMT. One day after the first infusion, a CTL response was detected in peripheral blood and this response increased in magnitude with each infusion. The infusion of a cumulative cell dose of  $1.47 \times 10^9$  cells/m<sup>2</sup> successfully reconstituted the CTL response in these deficient hosts to the same level as that detected in the healthy immunocompetent marrow donor (Figure 2).

The ability of adoptive immunotherapy to protect BMT recipients from CMV infection is likely to depend on the persistence of transferred CTL and Walter *et al* (1995) have evaluated the persistence of transfected CTL in these patients.<sup>43</sup> *In vivo* persistence of the adoptively transferred CTL clones was evaluated by using the unique

T-cell receptor (TCR) gene rearrangement of each clone as a genetic marker to detect the transferred cells *in vivo*. The TCR V $\beta$  and V $\alpha$  gene rearrangements in the infused clones were first identified by PCR using specific amplimers. CD8 CMV-specific CTL clones isolated from the patients at intervals after therapy were then similarly analysed to determine if the TCR expressed was the same as that in the infused clones. All of the CTL clones rescued from the patients up to 3 months after the infusion had an identical TCR gene rearrangement to one of the clones that was infused, confirming that adoptively transferred CTL were capable of persisting long term *in vivo*.<sup>43</sup>

Interestingly, the magnitude of the persisting response varied between patients; in the majority of patients, there was a decline in the magnitude of the CTL response 2–4 weeks after the infusions were discontinued. If the patient recovered a CD4 CMV-specific T-helper cell response on their own, the CD8 CTL response was maintained at levels similar to those seen in immunocompetent hosts. However, if patients did not recover CD4 CMV-specific T-helper cells, as was observed in three patients on high doses of prednisone to treat GVHD, the transferred CD8 CTL response declined to levels below those detected in immunocompetent hosts.<sup>42</sup> This was consistent with studies in animal models in which CD4 T-helper cells are required to sustain virus-specific CD8 CTL.<sup>44</sup> These results suggest that for durable immune reconstitution in patients on high-dose immunosuppression both CD8 CTL and CD4 CTL T-helper cell clones may need to be transferred or repetitive doses of CD8 CTL may be necessary.

The design of the Phase I study precluded a definitive analysis of the antiviral efficacy of CD8 CTL infusions. However, none of the patients in the Phase I study developed early- or late-onset CMV viraemia or disease, suggesting that additional study of this approach is warranted.

A Phase II study of high-risk BMT patients has been initiated to define the potential for adoptive immunotherapy with T-cell clones to prevent CMV disease. Patients

will receive two weekly infusions beginning at Day 28 to 35 after transplant and will also receive a single infusion of CD4 cells 2 days following the second CD8 infusion in an attempt to prevent the decline in CD8 responses seen in the Phase I study in those individuals who did not recover endogenous CD4 Th responses. Patients requiring high-dose prednisone therapy will receive repeat infusions of CD8 CTL on Day 62 post-transplant and CD4 on Day 64 after BMT. Because viraemia has a very high positive predictive value for disease and low levels of CMV antigenaemia have been detected in some patients with recovery of immune responses who are not at risk for CMV disease, viraemia and CMV disease will be the primary end-points for this study.

### *Late-onset CMV disease*

Additional study will be required to define the role for adoptive immunotherapy in the management of CMV disease in the BMT recipient. One potential application is in the prevention of late-onset CMV disease. The use of antiviral drug prophylaxis has reduced the incidence of CMV disease in the first 100 days after BMT to 3–10%.<sup>28,29</sup> However, long-term follow-up has revealed a disturbing increase in the probability of developing CMV disease after Day 100 from 4% in historical patients not receiving ganciclovir prophylaxis to approximately 20% in patients receiving pre-emptive or prophylactic ganciclovir. The mortality rate for these patients is in the range of 50%.<sup>30</sup> Risk factors associated with the development of late-onset CMV disease include acute and chronic GVHD and the use of ganciclovir within the first 100 days after transplant.

A possible explanation for this increased risk of late-onset CMV disease in ganciclovir recipients was provided by a study which analysed the recovery of CMV-specific T-cell responses in patients enrolled in a randomized, placebo-controlled study investigating the efficacy of ganciclovir prophylaxis administered in the first 100 days post-BMT. Patients in this study who had not developed CMV-specific CTL or T-helper cell responses before initiating ganciclovir therapy at Day 30 had a low probability of recovering these responses during ganciclovir therapy,<sup>27</sup> whereas patients in the placebo arm of the study who were deficient in CMV-specific CTL or T-helper cells at Day 30 were more likely to recover T-cell immunity to CMV (Table 8). Therefore, patients who received ganciclovir were more frequently deficient in CMV-specific T-cell immunity when ganciclovir therapy was stopped at Day 100 and would be predicted to be at risk of late reactivation and progression to disease. These results suggest that the restoration of T-cell immunity early after BMT may be beneficial for preventing both early- and late-onset CMV disease.

### *The potential for management of CMV with adoptive immunotherapy*

At the present time, cellular adoptive immunotherapy for CMV remains a research initiative. This area has proved informative for investigating the potential for adoptive immunotherapy to modulate immunity in humans for therapeutic benefit because of the clear correlation between the development of CMV disease and a deficiency in specific T-cell responses. Cell culture techniques have improved dramatically in recent years, and it is now possible to generate large numbers of cloned T-cells in relatively short periods of time. For example, once a T-cell clone is established it can be expanded from  $1 \times 10^5$  cells to more than  $10^{10}$  cells over two 10-day cycles of stimulation. These

	Placebo	Ganciclovir	P
Recovery of a CMV-specific CD8 CTL response	6/12 (50%)	1/9 (11%)	0.20
Recovery of a CMV-specific CD4 Th response	5/8 (63%)	1/5 (20%)	0.35
Number of deficient CMV-specific T-cell responses recovering	11/20 (55%)	2/14 (14%)	0.04

Table 8: Recovery of CMV-specific CD8 CTL and CD4 T-helper responses at Day 80–90 after BMT in patients deficient in these responses at Day 40<sup>27</sup>

improvements make the procedure less costly and feasible for larger scale studies. One attractive area for investigation is in the management of late-onset CMV disease, since this problem is unlikely to be effectively managed with antiviral strategies alone. As these patients are persistently immunosuppressed the only strategy that is likely to provide them with long-term protection is the reconstitution of immunity to CMV.

## Summary and Management Recommendations

The immunobiology of CMV should continue to be studied; this may eventually identify subgroups of patients at risk who should be monitored by surveillance.

*Research need*

### CMV vaccines

The search for a CMV vaccine has been underway since the link between this virus and cytomegalic inclusion disease was established more than three decades ago. Although clinical trials began in the 1970s with live virus vaccines, there are currently no licensed CMV vaccines available to the practising physician.

### Immunoglobulin immunotherapy

CMV immunoglobulin has a modest effect in prevention of severe CMV disease in renal transplant patients.

*Category 3 Recommendation*

In solid organ transplant patients, there is anecdotal evidence suggesting that antiviral therapy should be combined with immunoglobulin in D<sup>+</sup>R<sup>-</sup> groups. It is recommended that a formal study should be performed to address whether the combination of low risk or low intensity regimens in the D<sup>+</sup>R<sup>-</sup> patient is worth exploring with or without monoclonals or CMV immunoglobulin.

*Category 3 Recommendation*

There is no evidence from controlled trials that CMV immunoglobulin alone will significantly reduce disease in other solid organ transplant recipients. Immunoglobulin should not be used alone to reduce CMV infection, except in renal transplant patients.

*Category 1 Recommendation*

There is **no** evidence to show that immunoglobulin is superior to antiviral therapy. If immunoglobulin is used in any solid organ transplant setting, it should be combined with antiviral prophylaxis, or with monitoring that would allow pre-emptive therapy as it is not completely effective. Formal clinical studies are needed.

*Category 3 Recommendation*

Even though data are limited, there is evidence that antibody may have a clinical role in protecting against CMV disease. Any agent which reduces viral load even by a small amount (0.5–1.0 log) may nevertheless have an effect on CMV disease by reducing viral load to below a threshold level (see Chapter 1). For example, low potency antivirals, such as aciclovir, do not eliminate CMV infection but can still have a marked effect on CMV disease (see Chapter 2). It is possible, therefore, that immunoglobulin could have benefit in some patients.

*Category 3 Recommendation*

### T-cell adoptive immunotherapy

CMV infection has proved ideal for investigation of adoptive immunotherapy because of the clear correlation between the development of CMV disease and a deficiency in specific T-cell responses. There is potential for further clinical application of the technique, particularly as the problem of late-onset CMV disease is unlikely to be effectively managed with antiviral strategies alone.

As the cost of immunotherapy is less than using prophylactic ganciclovir from Day 30 to Day 100 post-transplant, expense will not necessarily be a prohibitive factor when considering this approach. Because the technique has potential for future treatment, the immunobiology of CMV should continue to be studied and the immune responses of patients should be monitored. This may help to evaluate the risk of, for example, late-onset CMV disease. It may also identify patients who should be monitored more closely by surveillance for reactivation, those who should be started on more aggressive regimes of antiviral therapy, or those who may be appropriate for investigational studies of T-cell therapy.

CMV disease occurs in T-cell immunodeficient individuals. For the practising physician, this means CMV disease is not normally found in a setting of lack of antibody responses. This could be dealt with using passive T-cell transfer, but non-T-cells, such as NK cells, may be effective.

## Human Herpesvirus Type 6 Infections in the Immunocompromised Host

Human herpesvirus type 6 (HHV-6) was first isolated in 1986 from patients with lymphoproliferative disorders by Salahuddin *et al.*<sup>1</sup> It was originally thought that the virus infected B-lymphocytes, but it is now known that HHV-6 mainly infects CD4 T-cells.

### The Natural History of Human Herpesvirus Type 6

#### *Virological aspects of HHV-6*

HHV-6 is a herpesvirus with the following characteristics:

- ◆ 162 kB linear double-stranded DNA
- ◆ Thymidine kinase (TK)<sup>-</sup>, DNA polymerase<sup>+</sup>
- ◆ Several glycoproteins (gB, gH)
- ◆ Lymphotropic (mainly T-cell)

Electron microscopy shows that HHV-6 is a typical herpesvirus; the virion has an envelope, capsid, core and tegument (Figure 1). The virus particle is 170 nm–200 nm in size, and the capsid size is around 100 nm.

HHV-6 can infect several cell types, including T-lymphocytes, B-cells, macrophages and human fibroblasts.

The HHV-6 genome has been completely sequenced.<sup>2</sup> The viral DNA contains two terminal repeat sequences (Figure 2).

HHV-6 can be subdivided into two variants, HHV-6A and HHV-6B. The two variants can be distinguished by immunological and molecular biological techniques, such as polymerase chain reaction (PCR). HHV-6A DNA has several deletions compared with HHV-6B; this allows the two variants to be distinguished on size. HHV-6A has been isolated mainly from individuals with AIDS. Figure 3 shows a phylogenetic tree of herpesviruses, based on the amino acid sequences of their respective gB proteins.

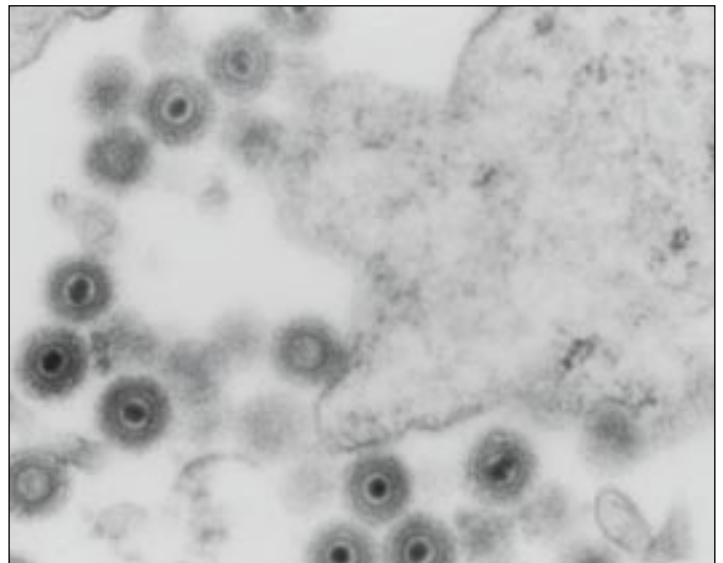


Figure 1: Electron micrograph of HHV-6 virus particles

#### *Prevalence of HHV-6 in healthy populations*

The prevalence of HHV-6 has been investigated in several populations. Throat swabs show that around 10% of babies aged 1–5 months excrete HHV-6B, and almost all children aged 2 years or older excrete HHV-6, mainly the B variant. Thirty per cent of adults secrete HHV-6 constantly from the throat (Figure 4).

# Chapter 4

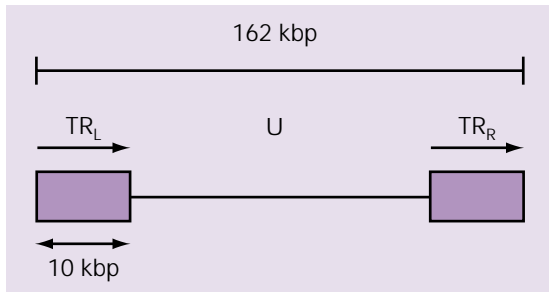


Figure 2: The genomic structure of HHV-6. TR, terminal repeat

HHV-6 is known to cause *exanthem subitum* or *roseola infantum* in infants (Figure 5). *Exanthem subitum* normally occurs in infants aged 6 months to 1 year. Symptoms include abrupt onset of fever lasting for a few days, and a normally mild rash which appears during defervescence.

In order to isolate the virus, heparinized blood was obtained from patients during the acute phase of *exanthem subitum*. The blood was separated by centrifugation on Ficoll hypaque and the monocytes were cultured. The cells were then examined microscopically. Staining these cells with sera from patients in the convalescent phase gave a positive result.

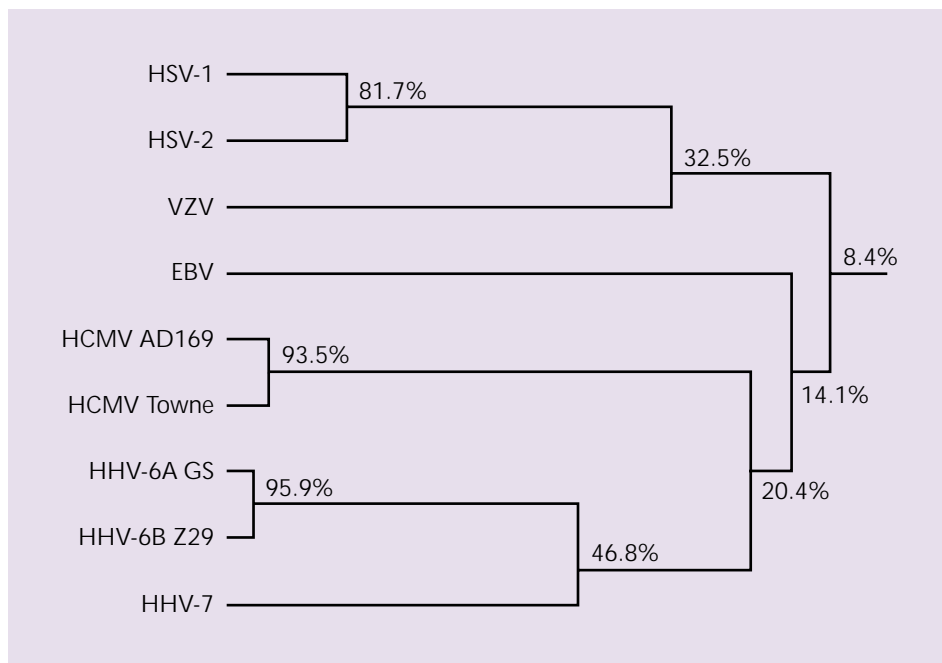


Figure 3: Phylogenetic tree based on human herpesvirus gB amino acid sequences

When attempts were made to isolate HHV-6 during the febrile phase of *exanthem subitum*, virus was isolated in 70% of patients. However, virus could be isolated from only 50% of patients in the rash phase. During the convalescent phase, no virus could be isolated. Similarly, no antibodies to HHV-6 were detected in the acute phase, but the antibody titre significantly increased in the convalescent phase.

These results, and previous studies from the 1950s, suggest that HHV-6 is the causative agent of *exanthem subitum*.

It is believed that HHV-6 is transmitted mainly through close contact, entering through the respiratory tract before systemic dissemination and organ invasion.

## Clinical manifestations of HHV-6

The clinical manifestations of HHV-6 infection have been evaluated in a total of 23 children with *exanthem subitum*. All patients had a fever and rash. Lymphadenopathy was present in around 85% of the children and 30% had hepatomegaly, 50% had

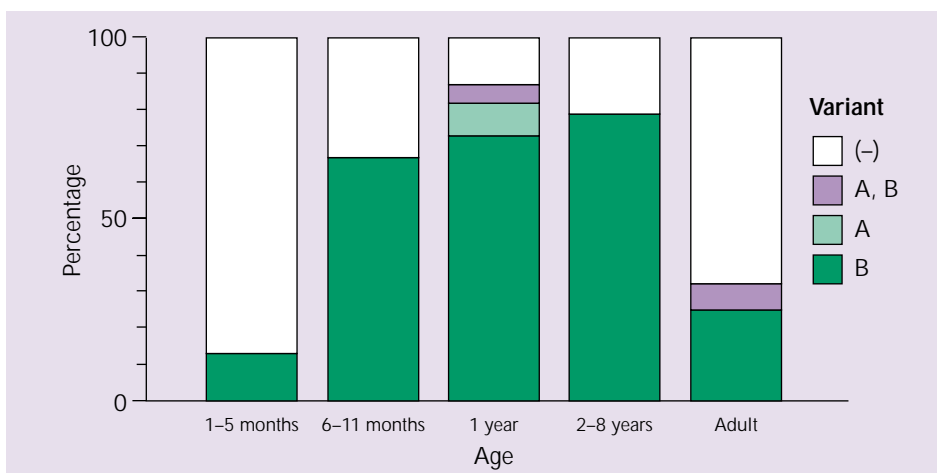


Figure 4: Detection of HHV-6 DNA from throat swabs in general populations

diarrhoea, and 10% had febrile convulsions. Although *exanthem subitum* is normally mild, a small proportion of patients will develop severe clinical symptoms. These include:

- ◆ Encephalitis<sup>3-5</sup>
- ◆ Liver dysfunction<sup>6</sup>
- ◆ Idiopathic thrombocytopenic purpura<sup>7</sup>

Investigations into the cause of encephalitis and encephalopathy by age showed that herpes simplex virus (HSV) was the causative agent in infants aged 0–6 months and HHV-6 was responsible for 18% of cases in the



Figure 5: Infant with exanthem subitum

7–12 month age group. HHV-6 DNA was detected in the spinal fluid of 9/10 patients in the convalescent phase of *exanthem subitum*, suggesting that the virus quickly invades the central nervous system (CNS) and may cause clinical symptoms such as febrile convulsions.

### Latency of HHV-6

HHV-6 has been isolated from the following tissues and organs, which may be the sites of latency or persistency:

- ◆ Salivary glands
- ◆ Kidney
- ◆ Lymph nodes
- ◆ Monocytes

In the case of monocytes, peripheral blood was collected from patients with *exanthem subitum* during both the acute and convalescent phases. Blood was fractionated into adherent and non-adherent cells. HHV-6 DNA was then detected by PCR. Acute phase blood samples contained HHV-6 DNA in both fractions, while convalescent phase samples contained HHV-6 DNA in the adherent cell fraction.

Monocytes collected from healthy adults were infected with HHV-6 *in vitro* and cultured. The presence of antigen was determined by immunofluorescence, and the

cells were also diluted and both tested for infectivity and analysed by PCR. Results confirmed that the cells were infected with HHV-6 and that the virus can replicate in the monocyte. Virus replicates for about 3 weeks after infection, but after that HHV-6 antigen could not be detected and no live virus was recoverable. However, HHV-6 DNA could still be detected in the monocyte.

These data suggest that the site of HHV-6 latency after primary infection may be the monocyte. However, *in vivo* experiments have failed to show that HHV-6 can be isolated directly from the monocyte. Quantitative PCR shows that most healthy individuals have low levels of HHV-6 DNA in their blood, although occasional persistence of high levels can occur.<sup>8</sup>

HHV-6 can be reactivated from individuals with AIDS, HTLV-1 carriers, lymphoma patients and leukaemia patients. HHV-6 can also be isolated from organ transplant patients and patients with chronic fatigue syndrome.<sup>9</sup> The viral burden of HHV-6 measured by quantitative PCR is significantly greater in organs at autopsy from individuals with AIDS than in controls.<sup>8</sup>

## The Immunopathogenic Role of Human Herpesvirus Type 6

### *HHV-6 in immunocompromised hosts*

*Exanthem subitum* is caused by HHV-6 variant B and not variant A. Variant A is more prevalent in populations with HIV infection, although it is unclear why. HHV-6 antibody titres have been measured in HIV-negative and HIV-positive African populations and results demonstrate that HHV-6 antibody titres are higher in individuals with HIV infection.<sup>10</sup> HHV-6 DNA has been detected in 21% of HIV-positive individuals, compared with an incidence of only 3.3% in HIV-negative individuals.<sup>11</sup> HHV-6 is found more frequently in patients with higher CD4 counts.<sup>12</sup>

### *Clinical symptoms associated with HHV-6 infection in AIDS*

Several reports describe the association between HHV-6 infection and disease in people with AIDS. It is unclear whether HHV-6 has a pathogenic role in individuals with AIDS. Corbellino *et al* (1993) described disseminated HHV-6 antigen in people with AIDS.<sup>13</sup> HHV-6 DNA was investigated by PCR in five individuals with AIDS and two controls. HHV-6 DNA was detected in the majority of tissues from the patients, including the CNS. Knox and Carrigan (1994) described nine individuals with AIDS who died from pneumonitis; HHV-6 DNA was detected in all 34 tissue samples from these patients.<sup>14</sup> In comparison, CMV was detected in only nine of the 34 samples. Dolcetti *et al* (1994) suggested that HHV-6B was associated with lymphadenopathy.<sup>15</sup> In HIV-positive patients, 12/18 were positive for HHV-6 DNA; 11 had variant B, one had both A and B. It is not clear whether there is a correlation between virus load and lymphadenopathy.

Encephalitis possibly caused by HHV-6 has been described by Knox *et al* (1995), Knox & Carrigan (1995) and McCullers *et al* (1995).<sup>16-18</sup> HHV-6A antigens were detected in astrocytes and neurones of infants with HIV. In the adult brain, the presence of HHV-6 was associated with some demyelination.

Finally, Qavi *et al* (1989) described co-infection with HHV-6 in the retina of individuals with HIV infection.<sup>19</sup>

HHV-6 may have a role in HIV replication. The HHV-6 immediate early (IE) gene can transactivate HIV LTR *in vitro*; however, cell culture experiments by other groups have suggested that HHV-6 may inhibit HIV replication.<sup>20-24</sup> It has also been shown that HHV-6 infects cells of immune system; these include:

- ◆ CD4<sup>25</sup>
- ◆ CD8<sup>26</sup>
- ◆ NK cells<sup>27</sup>
- ◆ Monocytes<sup>28</sup>
- ◆ Gamma and delta T-cells<sup>29</sup>

Although it is not yet clear whether HHV-6 has any role in the development of AIDS, there are associations between HHV-6 infection and disease in the HIV-positive individual.

## Impact of Antivirals on Human Herpesvirus Type 6

*In vitro*, ganciclovir shows the greatest potency against HHV-6, followed by foscarnet and then aciclovir, which has the lowest potency. The data on drug therapy for HHV-6 are very limited. There is some controversy over the effectiveness of antiviral drugs for the treatment of HHV-6 infections. Ganciclovir has been studied *in vitro* by two groups who report conflicting results. HHV-6 is not sensitive to aciclovir, perhaps because HHV-6 does not encode a TK necessary for the activation of aciclovir. There are no data on clinical responses of HHV-6 infections to drug therapy.

## Relationship Between Human Herpesvirus Type 6 and Multiple Sclerosis

A recent report has suggested that there may be an association between HHV-6 infection and the development of multiple sclerosis (MS).<sup>30</sup> Using representational difference analysis, the investigators detected a 341 bp fragment in a patient. Sequence analysis data suggested that the fragment was a DNA-binding protein of HHV-6. In the brain of 90% of both patients and the control group, HHV-6 was detected by PCR. However, HHV-6 antigens were found in oligodendritic cells and in neurones around MS plaques. This was not found in the control group. The authors postulate that HHV-6 is reactivated in the brain of patients.

## Human Herpesvirus Type 6 in Transplant Recipients

### *Bone marrow transplant recipients*

Carrigan *et al* (1991) described two cases of interstitial pneumonitis in bone marrow transplant (BMT) recipients which may have been associated with HHV-6 infection.<sup>31</sup> Pitalia *et al* (1993) also described eight cases of pneumonitis where HHV-6 DNA was detected.<sup>32</sup> Cone *et al* (1993) investigated HHV-6 in the lungs of 15 BMT patients compared with 15 healthy individuals and six fetuses.<sup>33</sup> Their results showed that although HHV-6 was detected, there was no relationship between viral load and disease.

One case report has been published suggesting that a case of encephalitis was due to HHV-6 infection of astrocytes.<sup>34</sup>

Three groups have described patients with rash and fever after BMT. Yoshikawa *et al* (1991) studied 25 BMT patients; HHV-6 could be isolated from the peripheral blood of 10 patients, and four of these patients had a rash.<sup>35</sup> Michel *et al* (1994) tried to detect HHV-6 DNA from skin biopsies.<sup>36</sup> Appleton *et al* (1994) described a relationship between graft versus host disease (GVHD) and HHV-6 reactivation.<sup>37</sup>

Finally, HHV-6 infection may have some influence on marrow suppression in BMT patients.<sup>38</sup>

## Renal transplant recipients

Four studies of HHV-6 infection in renal transplant recipients have been published. Morris *et al* (1989) studied 17 patients using immunofluorescent antibodies to HHV-6.<sup>39</sup> Four of the patients seroconverted after transplantation, and one of these developed a fever. Ten patients had antibody before transplant, and the titre of antibody significantly increased after transplant. Two of these patients had fever, but one of these was due to primary CMV infection.

Okuno *et al* (1990) described antibody response before and after transplant.<sup>40</sup> In 8/21 patients who were seropositive before transplant, there was a significant increase in antibodies within 2 months of the transplant. All of these eight patients showed symptoms of graft rejection.

Yoshikawa *et al* (1992) described 65 patients; HHV-6 was isolated from six of these patients after transplant.<sup>41</sup> In 36 of the 65 patients, an increase in HHV-6 antibody titre was observed. However, this group found no relationship between HHV-6 infection and graft rejection.

Jacobs *et al* (1994) described the case of one patient treated with OKT3.<sup>42</sup> There was an increase in IgM against HHV-6, and 13 weeks after transplant graft rejection occurred. The patient was treated with ganciclovir and the symptoms improved.

## Liver transplant recipients

There are two reports of HHV-6 infection in liver transplant recipients. Ward *et al* (1989) recorded a primary HHV-6 infection manifesting as hepatitis.<sup>43</sup> Sutherland *et al* (1991) monitored sera from 50 patients.<sup>44</sup> In this study, 30 patients were initially seronegative for HHV-6 antibodies and 13 of these seroconverted after transplantation. Of the 7 patients who had serological evidence of active HHV-6 infections but no evidence of CMV infection, four had fever, one had hepatitis, one had lung disease, and three had neurological disorders.

## Heart transplant recipients

Robert *et al* (1994) studied 58 patients with heart transplantation and hepatitis.<sup>45</sup> The authors could find no relationship between HHV-6 infection and hepatitis.

## Diagnosis of Human Herpesvirus Type 6 Infections

Three diagnostic strategies are available:

- ◆ Direct virus isolation is most frequently used, but has limitations
- ◆ Serological examination of IgM and IgG is possible using immunofluorescence, neutralization and ELISA
- ◆ PCR detection of HHV-6 DNA

Using measurements of IgG and IgM titres during the acute phase of *exanthem subitum* (ES), it is possible to show active infection (Figure 6).

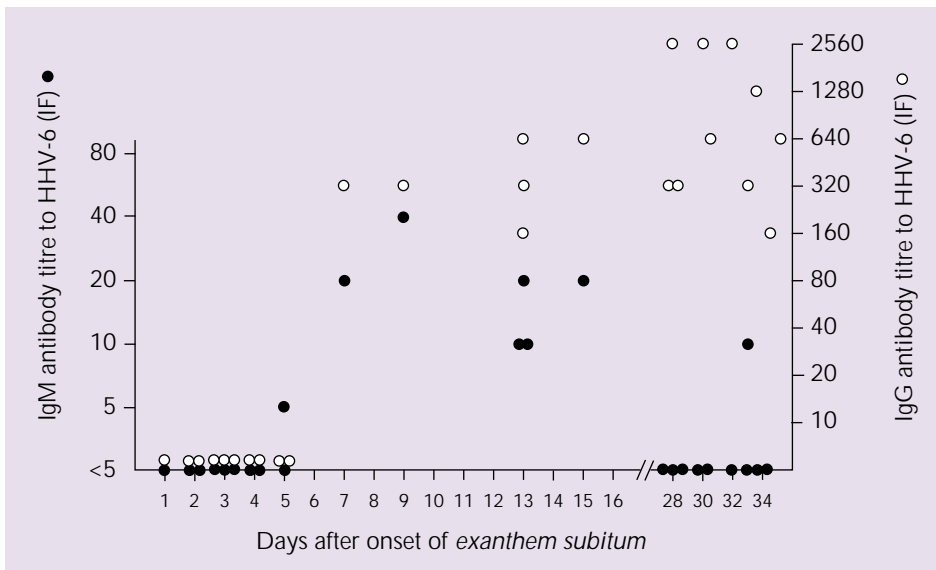


Figure 6: IgG and IgM antibody titres in exanthem subitum

HHV-6 DNA can be detected by PCR. Primers can be used against sequences encoding major capsid protein, gB or the IE region. This method can distinguish between HHV-6A and B variants. PCR can be performed on peripheral blood samples, cerebrospinal fluid (CSF) or throat swabs.

These methods have limitations for rapid diagnosis of HHV-6 infection:

- ◆ Virus isolation takes at least 10 days, and can take up to 1 month in samples taken from immunocompromised patients
- ◆ Serological examination is possible 5 days after onset of infection; this may be somewhat late in the course of disease to effect a treatment
- ◆ PCR may be subject to contamination

Because of these limitations, an indirect immunofluorescent test is suggested, using acute and convalescent phase sera.

## Summary and Management Recommendations

HHV-6, like CMV, is a common infection in general populations. In healthy individuals, infection with HHV-6 is unlikely to cause serious clinical manifestations in the majority of patients; however, in immunocompromised hosts, disease resulting from HHV-6 may be severe.

Physicians should consider HHV-6 infection in the differential diagnosis of encephalitis in transplant patients (especially if HHV-6 is detected in the CSF), pneumonitis and bone marrow suppression after BMT.

In individuals with HIV infection, HHV-6 may have a role in the pathogenesis of HIV.

As yet there are no recommendations for which method should be used for detection of HHV-6; methods currently available include direct virus isolation, an indirect immunofluorescent test, serological tests and PCR. All of these methods have limitations.

Data on the response of HHV-6 infection to antiviral treatment are both limited and unclear. No recommendations can yet be made on how HHV-6 infections should be treated.

## CHAPTER 1

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## Recommendation Categories

### *Category 1 Recommendation*

Consistent evidence from controlled clinical trials. For example, for an antiviral this would be two properly randomized controlled clinical trials. In the case of laboratory tests, consistent evidence from comparative studies.

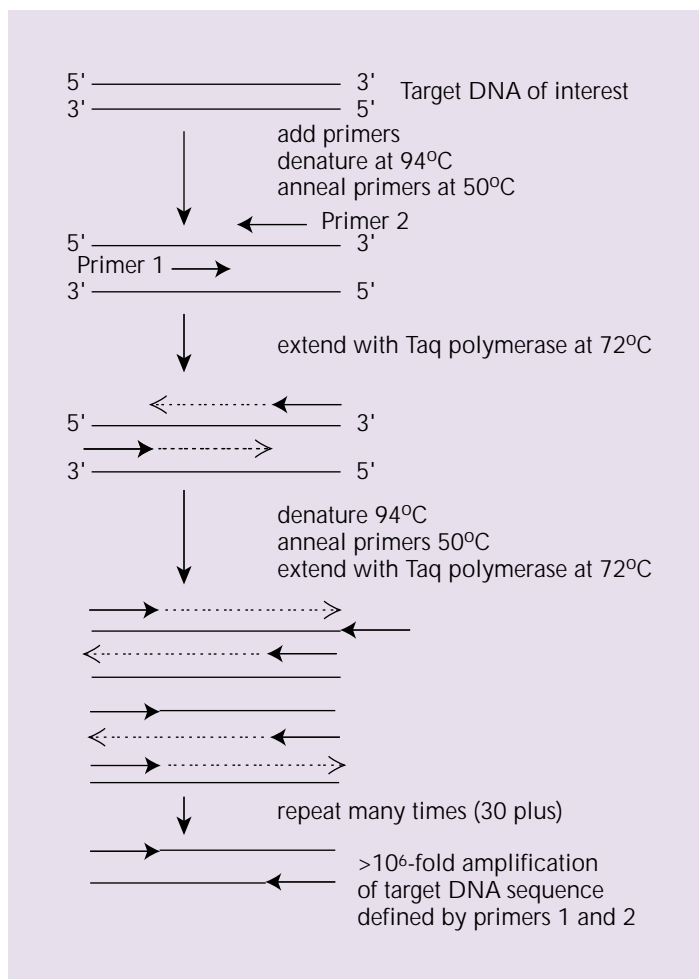
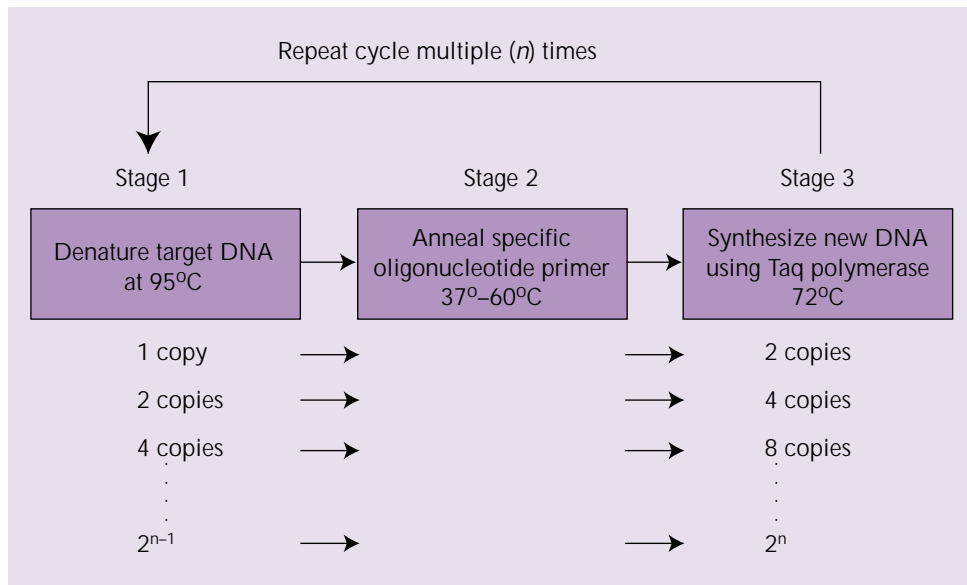
### *Category 2 Recommendation*

Evidence from at least one well-designed clinical trial without randomization, from cohort or case-controlled analytic studies (preferably from more than one centre), or from multiple time-series studies or dramatic results from uncontrolled experiments.

### *Category 3 Recommendation*

Evidence from opinions of respected authorities based on clinical experience, descriptive studies or reports of expert committees.

## Overview of the polymerase chain reaction (PCR)



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The *International Herpes Management Forum* (IHMF) World Wide Web site has information on the IHMF, forthcoming meetings and internet versions of *Management Strategies in Herpes*.

Internet address: <http://www.pps.co.uk/ihmf/welcome.htm>

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