Cytomegalovirus Infection Following Liver Transplantation: Review of the Literature

Souha S. Kanj, Ala I. Sharara, Pierre-A. Clavien, and John D. Hamilton

Cytomegalovirus (CMV) remains a major cause of problems following solid organ transplantation, accounting for a significant increase in morbidity and affiliated costs. Infection with CMV following orthotopic liver transplantation (OLT) is commonly seen as a result of marked cell-mediated immunosuppression and is an independent risk factor for opportunistic and fungal infections. The role of CMV infection in acute cellular or chronic rejection remains unclear. Recent advances in diagnostic modalities, particularly the use of the antigenemia assay and the polymerase chain reaction, have provided ways to quantitate viral load during infection or disease, as well as providing a useful marker of response to therapy. Ganciclovir remains the best antiviral agent for the treatment of CMV disease, but the use of combination therapy with other antivirals or CMV immunoglobulin may improve outcome for patients with severe disease. The ideal prophylactic therapy for patients undergoing OLT remains to be identified, as tested regimens have shown variable efficacy when analyzed with regard to defined risk groups. The use of risk group-specific prophylaxis may prove to be most successful, however, in terms of efficacy and cost savings. Future advances in basic CMV virology and transplant immunology will be essential in defining rational approaches to control and prevention of CMV infection and disease following liver transplantation.

Cytomegalovirus (CMV) is the single most important opportunistic pathogen following solid organ transplantation, resulting in considerable morbidity, prolonged hospitalization, and increased affiliated costs [1–4]. The incidence of CMV infection among patients undergoing liver transplantation ranges from 23% to 85%, and symptomatic disease develops in 15% to 40% of these patients [3, 5]. In addition to causing various clinical infectious syndromes, CMV has direct immunosuppressive properties, and infection with CMV is an independent risk factor for bacterial and fungal superinfection [6–10]. Furthermore, it has been suggested that CMV plays a role in hepatic allograft rejection [11].

In view of the significant impact of CMV on solid organ transplantation, and because of a constantly expanding list of potential transplantation candidates and the large number of previously CMV-infected individuals in the general population (hence, in the donor pool) [12], there is a need for reliable and cost-effective prophylaxis against and treatment for CMV infection. Despite recent progress in diagnosis and treatment, the ideal prophylactic regimen after liver transplantation remains to be defined, particularly for high-risk patients. Findings of studies of prophylaxis against CMV following other solid organ transplantations may not be applicable to clinical liver transplantation, and caution is needed in extrapolating these conclusions. This article reports on the approach to and management of CMV infection in liver allograft recipients, with particular emphasis on advances in diagnosis and prevention.

Epidemiologic Characteristics

CMV is a ubiquitous, double-stranded DNA virus belonging to the Herpesviridae family. It frequently infects humans and spreads by direct contact through exposure to infected secretions, by blood transfusion, transplacentally, or following organ transplantation. Seropositivity rates in the general population vary from 30% to 70% in developed countries (approaching >70% in older populations and in lower socioeconomic groups) and are near 100% in developing countries [12]. Three potential sources of CMV infection following solid organ transplantation have been identified: (1) the donor organ, (2) transfused blood products from a seropositive donor, and (3) reactivation of endogenous virus [13, 14].

Like other members of the herpesvirus group, CMV has a propensity to cause latent or chronic infection and to spread by cell association. These properties contribute importantly to both the epidemiology of CMV and the need for cell-mediated immunity in controlling infection. CMV rarely causes disease in the immunocompetent host, although spontaneous presentations of CMV disease as a mononucleosis-like syndrome with or without multiorgan involvement (including infection of the heart, lungs, blood, skin, gastrointestinal tract, and CNS) have
been described [15]. On the other hand, liver transplant recipients experience a considerable decline in cell-mediated immunity following initiation of immunosuppressive therapy. Consequently, in these patients, CMV infections frequently can be symptomatic and occasionally are fatal.

**Patterns of CMV Infection**

Depending on pretransplantation CMV status, three distinct patterns of CMV infection are seen after liver transplantation: primary infection, reactivation, and superinfection. Primary infection occurs when a CMV-seronegative patient receives a liver allograft from a seropositive donor or receives a transfusion with blood products from seropositive donors. Reactivation of CMV occurs when a seropositive recipient receives a liver from a seronegative donor and then latent virus reactivates because of either immunosuppression or the allogeneic antigenic stimulus. Superinfection is the result of replication of a new CMV strain acquired by organ transplantation or blood transfusion in an already-seropositive recipient. Although viral DNA sequencing analysis may help differentiate reactivation from superinfection, these infections are clinically indistinguishable [16, 17]. However, studies of renal transplant recipients have suggested that, on the basis of outcome, increased severity of disease is associated with superinfection [18, 19].

**Risk Factors for the Development of CMV Infection**

Several variables are associated with an increased frequency of CMV infection in liver transplantation patients. The single most important risk factor for the development of CMV infection is the serological status of the donor and recipient [3, 5, 20, 21]. Other risk factors include age, increased perioperative blood transfusion requirements, use of antilymphocyte therapy, retransplantation for acute rejection, and fulminant liver failure as an indication for transplantation [2, 3, 5, 20–22]. The level and type of immunosuppression are also significant determinants of disease and its severity. Antilymphocyte preparations such as OKT3 (Orthoclone, Raritan, NJ) are very potent reactivators of CMV, while cyclosporine, corticosteroids, and rapamycin are poor reactivators but cause an increase in viral replication once the virus is activated [23].

To understand the role of CMV in liver transplantation and to compare the efficacy of therapeutic and prophylactic regimens, it is important to review the parameters used in defining the spectrum of illness with CMV. CMV infection is defined as viral shedding or seroconversion in a patient who was seronegative pretransplantation. Infection does not always result in symptomatic disease with end-organ dysfunction, but factors associated with progression to symptomatic disease include primary CMV infection and use of antilymphocyte therapy [24, 25]. Patients whose conditions progress to CMV disease may present with a distinctive viral syndrome and localized or disseminated disease. The CMV viral syndrome is manifested by fever, leukopenia, thrombocytopenia, and—rarely—atypical lymphocytosis, in addition to a positive viral culture [5]. Localized disease is defined as histopathologic evidence of invasion of a single organ, with or without a positive viral culture of the involved tissue. Disseminated disease is defined as tissue involvement of two or more noncontiguous organ sites. The presence of CMV pneumonia or disseminated CMV disease is indicative of severe CMV disease [26].

It is unclear what predisposes a particular patient to have disseminated disease and another patient with presumed similar risk factors to remain asymptomatic. Factors such as TNF-α may be important in regulating the balance between latency and reactivation of CMV infection because of their ability to stimulate the activity of the CMV intermediate-early enhancer/promoter region in culture [27, 28].

**Clinical Manifestations**

In the absence of prophylaxis, the incidence of CMV infection after liver transplantation ranges from 23% to 85%, and in approximately one-half of these patients clinical disease develops [1, 3, 5, 29–31]. Rates of CMV infection are determined by a variety of factors, including the CMV serological status of donor and recipient, the intensity with which the diagnosis is pursued (e.g., the monitoring of viral shedding), the net state of immunosuppression, and the prophylactic regimen or preventative methods used. In a report on 218 liver transplant recipients at the Mayo Clinic by Marin et al. [5], it was noted that CMV infection occurred in 53% of patients and CMV disease in 25%, with the incidence directly related to the donor/recipient CMV serological status at transplantation (figure 1). Seronegative recipients of an organ from a seropositive donor had the highest risk of infection and disease (77% and 61%, respectively), whereas the lowest risk (10%) was associated
with a seronegative status of both donor and recipient. Seropositive recipients had an intermediate risk, ranging from 49% to 66% for CMV infection and 10% to 23% for CMV disease. The incidence of CMV infection and disease in the latter group was higher when the organ was from a seropositive donor, suggesting a probable risk of CMV superinfection in addition to that of viral reactivation.

The timing and the clinical manifestations of CMV infection after liver transplantation are similar to those following other solid organ transplantation. The median time to onset of CMV infection is 28 days (range, 2 weeks to >5 months), and CMV disease occurs ~2 weeks after the onset of viral shedding (a median of 43 days after transplantation) [2, 32–35]. Disease symptoms can be diverse, from mild to life-threatening, and the involvement of almost any organ is possible. Fever is the most common manifestation and occurs in approximately two-thirds of patients [34]. A mononucleosis-like syndrome with malaise, anorexia, myalgias, arthralgias, and hematologic abnormalities is occasionally seen. The latter include leukopenia, thrombocytopenia, and the presence of atypical lymphocytes on the peripheral smear [36]. The allograft is the most common site for organ involvement, and CMV hepatitis has been noted in 17% of liver transplantation patients and in up to 50% of patients with primary infection (seronegative recipients from seropositive donors) [5, 33, 35, 37].

Biochemical evidence of CMV hepatitis consists of a non-specific elevation of aminotransferase levels or a cholestatic pattern with elevations in serum bilirubin, γ-glutamyltransferase, and alkaline phosphatase values, but the diagnosis requires a liver biopsy [35]. The usual findings on biopsy include microabscesses or microgranulomata scattered around the liver lobule and an occasional infiltrate of chronic inflammatory cells in the portal triads (figure 2) [38]. Other organs that can be involved are the lungs, the gastrointestinal tract, and the retina (usually >6 months posttransplantation).

CMV pneumonia occurs in 13%–30% of patients after liver transplantation and is associated with considerable morbidity and mortality [3, 5, 39]. CMV pneumonia can present with dyspnea; tachypnea followed by a hacking, nonproductive cough; and hypoxemia. Radiographically, the disease is typically symmetrical, with bilateral and interstitial involvement (often with a nodular component) usually beginning in the lower lobes and progressing inward and outward to involve the whole lung, resulting in respiratory failure [40]. The gastrointestinal tract is affected in a large number of patients, but clinical, symptomatic disease is seen in only 5% of cases [3, 5, 41]. Gastrointestinal involvement is manifested by gastric, duodenal, or colonic ulcerations leading to nausea, vomiting, early satiety, hemorrhage, and (rarely) peritonitis secondary to viscus perforation [42]. Involvement of the skin, heart, or CNS is very rare [43].

In addition to its direct pathogenic effect, CMV is a predisposing risk factor for bacterial and fungal infections following liver transplantation [6–10]. CMV infection has direct immunosuppressive effects on the host’s immune system [44] and is associated with reduced lymphocyte response to antigens and mitogens [6, 45], an inversion in the ratio of helper to suppressor T cells [46–48], and suppression of monocyte and natural killer cell functions [49]. Indirect effects on the immune system related to dysregulation of the cytokine network have been reported [50–53].

It has been suggested that CMV plays a role in allograft rejection [54–58]. The in vitro effects of CMV on cell-mediated immunity include immune activation with increased release of IFN-γ from infected cells, upregulation of cytotoxic T-cell response, and induction of class I and class II histocompatibility leukocyte antigens (HLAs) on infected cells [56, 59–61]. CMV infection has been associated with accelerated atherogenesis in cardiac transplant recipients and with bronchiolitis obliterans in patients undergoing lung transplantation [62–65].

Reports on liver transplantation patients describe CMV infection as an independent risk factor for the development of the vanishing bile duct syndrome, a manifestation of chronic rejection [11]. It has been suggested that an HLA-DR match between donor and recipient increases the incidence of CMV hepatitis and possibly accelerates the development of chronic allograft rejection, perhaps through HLA-DR-restricted immunologic mechanisms directed toward CMV viral antigens [66]. These findings, however, have not been confirmed in other studies [67, 68], and the causative link remains questionable.

The association of CMV with acute cellular rejection in liver transplantation is controversial. Theoretically, CMV infection may predispose to cellular rejection in that there is often a need to decrease immunosuppressive therapy in the setting of CMV infection. Conversely, the need for increased immunosuppression to treat cellular rejection may predispose to CMV infec-
tion. Although retrospective studies have suggested that CMV prophylaxis has a beneficial effect on the incidence of acute cellular rejection [69], a direct cause and effect remains to be confirmed.

Despite all the effects of CMV infection on the liver transplantation patient, the overall impact appears to be an increase in morbidity with no significant effect on graft or patient survival. In a study at the University of Nebraska by Stratta et al. [3], the overall survival rate among 79 patients with CMV disease was not significantly different from that among 189 patients without CMV disease up to 4 years from transplantation, although there was a trend toward increased mortality among patients with CMV disease. High awareness of the disease, the institution of prompt effective therapy, and the careful management of immunosuppression are likely to limit morbidity and mortality related to CMV disease in patients following liver transplantation.

Diagnostic Methods

Because of the considerable impact of CMV infection on the liver transplant recipient, rapid and specific methods of diagnosis are necessary. The ideal method should be reliable, widely available, and inexpensive. It should accurately provide a way to establish the diagnosis early in the course of infection or disease so as to guide specific antiviral therapy and allow judicious management of immunosuppression and monitoring of graft function. An understanding of currently available diagnostic methods is essential for the proper selection and use of therapeutic and preventative measures. For example, screening strategies that allow the diagnosis of CMV infection at an early stage (e.g., the antigenemia assay) may be useful in predicting CMV disease and may provide a rationale for short preemptive therapy. On the other hand, methods that confirm the diagnosis of CMV disease associated with tissue invasion (e.g., liver biopsy for CMV hepatitis) would dictate an immediate need for specific and prolonged antiviral therapy to prevent disease progression and its associated morbidity and mortality.

Serological testing. CMV serology is useful for pretransplantation assessment of the recipient’s risk of CMV infection because of the increased risk associated with donor and recipient seropositivity [3, 5, 20, 21]. In general, the development of CMV IgM antibodies or a fourfold rise in IgG titer over time indicates acute infection. Although these tests are potentially helpful in confirming primary or secondary CMV infection, they are highly insensitive in immunocompromised patients and have limited clinical usefulness because of the prolonged time for confirmation of diagnosis [70, 71]. Moreover, CMV IgM can be detected in cases of asymptomatic reactivation, and a test for it may be falsely positive in the setting of positive rheumatoid factor or infection with other herpesviruses such as Epstein-Barr or herpes zoster virus [70, 72–74].

Viral cultures. Isolation of CMV from tissue or body secretions remains the “gold standard” against which other tests are compared. Conventional viral culture methods require a long time (1–2 weeks) to confirm the viral cytopathic effect on fibroblast cultures, and viral recovery may be further delayed when viral replication is low [71]. The development of the shell vial assay, however, has permitted the detection of viral infection in as early as 12–24 hours. This method uses a monoclonal antibody to detect a 72-kD major immediate-early antigen of CMV in urine, blood, or throat cultures performed on flat monolayers on coverslips in shell vials [75–77]. This assay is widely available, rapid, and specific for CMV infection. Sensitivity is diminished, however, in the case of blood cultures because of the toxicity of leukocytes to both the virus and the fibroblast monolayer [78].

Histopathologic diagnosis. Histologic diagnosis of tissue-invasive CMV disease is based on the presence of characteristic intranuclear inclusions (Cowdry type A) in enlarged cells with a prominent nuclear rim, giving the typical “owl’s eye” appearance (figure 2). Endothelial cells are characteristically involved, with resultant microthrombi, but epithelial involvement is not uncommon. These findings are focal in nature, and confirmation of diagnosis is therefore subject to sampling error. Sensitivity and specificity are enhanced by complementary use of immunostaining and in situ hybridization [79].

Antigenemia assay. Grefte et al. [80] have recently developed an immunoperoxidase assay using monoclonal antibodies, directed against a 65-kD structural late viral protein, to demonstrate CMV antigens in peripheral blood leukocytes. CMV antigenemia is synonymous with CMV infection and can be detected as early as 1 week prior to the onset of disease. The number of positively stained leukocytes appears to be an indicator of viral load and disease severity as well as a helpful marker for the monitoring of infection and the patient’s response to therapy [81, 82].

In solid organ transplantation, a small number of antigen-positive cells (<10 per 50,000 polymorphonuclear cells) generally indicates asymptomatic infection, whereas a large number (>50 antigen-positive cells per 50,000 polymorphonuclear cells) indicates a 60% likelihood of CMV disease [83]. The positive predictive value of the antigenemia assay in detecting disease, however, is not absolute, meaning that some patients with low antigen levels may have severe disease and that patients with high antigenemia levels may remain asymptomatic. Despite some limitations, the antigenemia assay, alone or in combination with shell vial cultures, deserves serious investigation for its value in monitoring for CMV as a guide to preemptive therapy in cases of liver transplantation. Standardization of the assay is needed, however, to allow for comparative interpretation of studies at different transplantation centers.

Nucleic acid amplification. PCR has been used as a rapid diagnostic technique for CMV infection because of its extreme sensitivity and specificity in detecting viral DNA [84–86]. Its ability to detect very few DNA copies raises concern, however, that a positive signal may not differentiate between a replicating and a latent virus. These limitations may be eliminated by the
use of more stringent conditions, more appropriate primers, and a lower number of amplification cycles for quantitation of viral load [87]. PCR is similar to the shell vial culture and antigenemia assay in that a positive PCR for CMV DNA is indicative of viral replication but is not diagnostic of CMV disease [88].

Reverse transcriptase-PCR (RT-PCR) has the advantage of specificity for active viral replication because it selectively detects viral mRNA transcripts (coding for structural and non-structural proteins) in peripheral blood leukocytes [89]. Preliminary results from our institution suggest that it compares favorably with shell vial culture in its rapidity, sensitivity, and specificity when applied to bronchoalveolar lavage samples from lung transplant recipients. A recent report by Patel et al. from the Mayo Clinic found RT-PCR of peripheral blood mononuclear cells to be less sensitive but more specific than the shell vial culture and PCR in diagnosing CMV disease [90]. It is unclear if quantitative RT-PCR of viral mRNA transcripts can serve to predict whether a given infection will progress to symptomatic disease.

Miscellaneous methods. Urine β2-microglobulin is a rapid and sensitive indicator of infection with a lymphotropic virus [91]. Similarly, elevated serum levels of soluble interleukin-2 receptor have been described to be indicative of viral infections in liver transplantation patients and to even antedate clinical infection [92]. Both tests, however, lack specificity for CMV infection and consequently are of limited clinical use.

On the basis of the discussion above, we can conclude that PCR of serum is most sensitive in the diagnosis of CMV infection, while RT-PCR is most specific for disease. The antigenemia assay is highly sensitive for CMV infection, and the level of antigenemia is helpful in monitoring response to therapy. Viral blood cultures, however, remain the most widely used method for the diagnosis of CMV infection because of limited availability of the antigenemia assay and PCR and the need for standardization of the quantitative methods.

Treatment of CMV Disease

The various treatment approaches against CMV in the liver transplant recipient hinge on the differentiation between CMV infection and disease. For CMV infection, evidenced by the presence of viral shedding, therapy is aimed at preventing clinical disease. This so-called preemptive therapy is beneficial in a subset of patients and is discussed under prophylaxis (see next section). On the other hand, for CMV disease evidenced by the CMV viral syndrome or tissue invasion, therapy should be prompt, effective, and aimed at limiting disease severity and its associated morbidity and mortality.

The development of ganciclovir has revolutionized the treatment of CMV disease in immunocompromised patients. Ganciclovir, an acyclic deoxyguanosine analog, is activated to the triphosphate moiety preferentially in infected cells, where it functions as an inhibitor and a fault substrate for CMV DNA polymerase [93]. Despite the lack of controlled trials, ganciclovir is reported to produce favorable clinical responses in up to 80% of immunocompromised patients with CMV disease, including liver transplant recipients, and is considered the first-line agent of treatment in the management of established CMV disease [94-99]. In a study at the University of Nebraska, Stratta et al. reported on their experience with ganciclovir in the treatment of CMV disease in liver transplant recipients over a 5-year period [100]. A total of 132 episodes of CMV disease (103 initial episodes and 29 relapses, including 22 cases of primary CMV disease) were treated with ganciclovir at a dosage of 5 mg/kg twice daily for a mean duration of 16 days. Disease response, defined by clinical improvement and negative viral cultures, was documented in 74 cases (71.8%). CMV disease recurred in 21 patients (20.4%) at a mean time of 33 days after completion of ganciclovir therapy and was successfully retreated with ganciclovir in 14 patients (66.7%). No cases of ganciclovir-resistant CMV disease were documented.

Recurrence and relapse of CMV infection are less common in patients undergoing liver transplantation than in patients with AIDS [101-103] and do not necessitate maintenance therapy with ganciclovir. One manifestation of CMV disease, CMV retinitis, deserves special consideration. This uncommon manifestation typically occurs late (>6 months from transplantation) but is associated with considerable morbidity, causing complete and irreversible loss of vision if left untreated. Information about experience with management of CMV chorioretinitis in liver transplant recipients is anecdotal. However, on the basis of the extensive experience with regard to patients with AIDS, active retinitis can be expected to recur in most if not all patients because ganciclovir is virustatic and not viricidal. Therefore, maintenance therapy with ganciclovir should be strongly considered while these patients are under heavy immunosuppression. Such therapy is not uniformly effective, however, and breakthrough infections with progressive CMV retinitis in patients with AIDS have been described [102, 103]. Resistant strains of CMV with mutations in the viral DNA polymerase gene have been recovered from immunosuppressed patients receiving long-term therapy with ganciclovir [104, 105].

Ganciclovir is used in an intravenous form because of its poor availability after oral administration [106]. Administration of oral ganciclovir to liver transplant recipients has not been reported, but the current limited experience with it as maintenance therapy for CMV retinitis in patients with AIDS has been promising [107]. Clearance of ganciclovir is dependent on renal elimination, and dose adjustment is necessary for patients with renal insufficiency [108]. Recommended dose reductions of intravenous ganciclovir are based on creatinine clearance, as follows: 50-80 mL/min, 2.5 mg/kg every 12 hours; 25-50 mL/min, 2.5 mg/kg every 24 hours; and <25 mL/min, 1.25 mg/kg every 24 hours. Blood levels of ganciclovir are decreased by 50% after 4 hours of hemodialysis, hence the need for readministration shortly after dialysis [109]. Neutropenia is the most important dose-limiting adverse effect [93]. Other side
effects include hepatocellular dysfunction, thrombocytopenia, infusion site reactions, and gastrointestinal abnormalities [93, 110].

Foscarnet (trisodium phosphonoformate) is a noncompetitive viral DNA polymerase inhibitor with established activity in the treatment and prevention of CMV retinitis in patients with AIDS [93, 111]. Experience with this drug in cases of solid organ transplantation is limited, and this drug is currently reserved for patients with CMV disease who are intolerant of ganciclovir therapy. Adverse effects include nephrotoxicity, seizures, electrolyte abnormalities, and gastrointestinal disturbances [112]. Treatment with foscarnet costs approximately three times that with ganciclovir.

Combination therapy including ganciclovir and CMV hyperimmune globulin (CMVIG) has been shown to improve survival after CMV pneumonitis in allogeneic bone marrow transplant recipients [113]. Proposed mechanisms of increased efficacy include the ability of antibodies to neutralize CMV and block CMV-specific cytotoxic T-lymphocyte effector cells, thereby modifying the immunologic response and resultant tissue damage [114, 115]. In the murine CMV model, treatment with the combination of CMVIG and antiviral agents results in higher survival rates than those associated with either modality alone [116].

To assess the role of combination therapy with ganciclovir and CMVIG in the treatment of CMV pneumonia in liver transplant recipients, the Boston CMVIG study group [117] performed a retrospective analysis of 17 orthotopic liver transplantation patients with CMV pneumonia: 6 patients were given ganciclovir monotherapy, and 9 patients received ganciclovir in addition to intravenous CMVIG (100 mg/kg, on alternate days) for 14 days. In the combination therapy group, the mean duration of ventilator-dependent respiratory failure was significantly reduced from 21.3 days to 10.8 days, and there was a trend toward improved survival at 1 year. Despite the study limitations, combination therapy appears beneficial in patients with disseminated or severe CMV disease, defined by multiorgan involvement or the presence of CMV pneumonia or invasive fungal disease in the setting of CMV infection [118].

A cornerstone in the treatment of CMV disease in solid organ transplant recipients is the judicious use of immunosuppressive therapy. Prior to use of ganciclovir, treatment modalities necessitated a reduction in immunosuppression, which in turn caused an increased risk of graft rejection. Currently, there does not appear to be a need for reducing immunosuppressive therapy when ganciclovir is used, although some investigators advocate reducing or discontinuing use of azathioprine by patients receiving triple-immunosuppressive therapy [119]. The use of ganciclovir for CMV disease allows for baseline immunosuppression to be maintained and for allograft rejection to be treated with pulse steroids and even with potent antilymphocyte therapy [120]. For concomitant CMV disease and steroid-resistant rejection necessitating the use of OKT3, ganciclovir therapy should be extended for 5 to 7 days after the course of OKT3 is completed.

**Prevention of CMV Infection**

Despite the availability of effective treatment for CMV disease, such therapy has not been shown to reduce the secondary effects of CMV infection, including its immunomodulatory action and the resultant risk of bacterial and fungal superinfection. In fact, these effects are felt to antedate clinical CMV disease, hence the argument for early intervention or prophylaxis [121]. Because of the significant impact of CMV on liver transplantation, efforts aimed at prophylaxis against CMV infection or disease have received considerable attention. It is important to note that prophylactic regimens must be critically evaluated in the context in which they are studied. In other words, the patients' CMV risk status, the type of organ transplanted, and the case definitions used for CMV disease are important when one considers the applicability of a defined regimen.

In the transplantation literature, prophylactic regimens against CMV abound [69, 122–129], but the rationale for their use is based predominantly on retrospective studies or on the local experience of a particular transplantation center. There is no consensus about which prophylactic regimen is preferable, largely because of the small number of randomized trials (table 1) and the vast differences in study designs, subgroup risk stratifications, and immunosuppressive regimens used. Additional confounding factors include the variability in methods used for monitoring CMV infection and the timing and intensity with which they are carried out.

**Protective matching of donor and recipient.** Protective matching, or transplanting an organ from a seronegative donor into a CMV-seronegative recipient, has been shown to be associated with a decrease in both the incidence and the severity of CMV disease [134]. Because of an increasing shortage of donor organs and the often long waiting time for transplantation, allocation of livers on the basis of CMV-serological compatibility has not been implemented in most transplant centers.

**Blood product screening.** The risk of transfusion-acquired CMV infection is proportional to the number of units transfused. Early blood bank studies involving primarily immunocompetent blood recipients suggested a risk of 2.4%–12% per unit [135, 136], while more recent studies have shown the risk to be <1% [137, 138]. The risk of infection is decreased with use of cryopreserved blood or leukocyte-poor blood (CMV is latent in leukocytes). In a study involving liver transplant recipients, symptomatic primary CMV infection occurred in 16% of CMV-seronegative patients who received organs from seronegative donors [130]. Avoiding blood products from CMV-seropositive donors and using leukocyte-filtered blood products have therefore been widely advocated. This approach is attractive particularly when applied to donor negative–recipient negative matches because the cost of diagnosing and/or treating a primary CMV infection is eliminated. We currently provide up to 20 units of packed RBCs from CMV-seronegative donors for liver transplant recipients with a seronegative pre-
transplantation status. The extent of the effect of such a transfusion policy on the incidence of superinfection in seropositive recipients is unknown.

**Active immunization.** A placebo-controlled trial involving immunization of renal transplant recipients with the live attenuated Towne vaccine revealed a significant reduction in the severity of CMV disease in CMV-seronegative patients who received a kidney from a seropositive donor [139]. However, there were no differences between the treatment groups in terms of the rates of CMV infection or disease, and the vaccine failed to prevent superinfection with other human CMV strains. The use of an effective subunit vaccine may be a safer and more promising alternative in the future.

**Passive immunization.** CMVIG containing high titers of antibodies to CMV antigens provides effective prophylaxis against CMV in kidney transplant recipients, resulting in a reduction in the incidence of symptomatic CMV disease [26]. Retrospective and prospective studies suggest that the administration of immune globulin to liver transplant recipients can be protective against CMV, when used alone or in combination with ganciclovir or acyclovir [69, 130, 140]. In a randomized, double-blind, placebo-controlled trial involving 234 liver transplantation patients, Snydman et al. [130] reported on the use of CMVIG: prophylaxis (150 mg/kg of body weight) was given within 72 hours of transplantation; repeated doses were given at 2, 4, 6, and 8 weeks post-transplantation; and 100 mg/kg was given at 12 and 16 weeks. CMVIG therapy was associated with a decrease in the overall incidence and severity of CMV infection and disease in seropositive patients but not in high-risk patients, namely CMV seronegative recipients of organs from seropositive donors. The lack of a demonstrable protective effect in the high risk group may have resulted from the small number of these patients in each group (fewer than 20 in each arm) and the increased use of antilymphocyte therapy in the CMVIG arm in comparison with that in the placebo group (29 vs. 20 patients, respectively).

In an ensuing report [141], the same group of investigators reported on an open-label study of CMVIG in 21 seronegative liver transplant recipients enrolled after completion of the random-assignment trial: 9 received an organ from a seropositive donor and 12 from a seronegative donor. Among the 12 seronegative recipients of a seronegative organ, only 3 received CMV-seronegative blood products, and only 1 CMV infection occurred. Among the 9 seronegative recipients of a seropositive organ, 6 (67%) had CMV infection and 3 of the 6 developed CMV disease, all in the form of mild CMV hepatitis.

In summary, prophylactic CMVIG is effective in the prevention of CMV disease in low- and intermediate-risk patients, as stratified by pretransplantation CMV-serological status. An important consideration is the issue of the cost-benefit ratio of this therapy (a course of CMVIG costs ~U.S.$6,000–$12,000) if it is widely applied to all transplantation patients, including those at low risk. Any cost-effectiveness comparison should, however, also take into consideration the reported reduction in the incidence of fungal and opportunistic infections with the use of CMVIG in cases of solid organ transplantation.

**Antiviral agents.** In a randomized, placebo-controlled trial of high-dose acyclovir administered for 12 weeks to renal transplant recipients, Balfour et al. [142] reported a significant decrease in the incidence of CMV disease, with the strongest protection provided to seronegative recipients of seropositive organs. Results in cases of liver transplantation, however, have been disappointing in comparison with those of other prophylactic regimens, particularly for high-risk patients [131–133].

Administration of ganciclovir, followed by that of high-dose acyclovir (0.8–3.2 g daily), was found to be effective in reducing the incidence of CMV infection and of symptomatic CMV

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Table 1. Data from randomized trials of prophylaxis against CMV infection following orthotopic liver transplantation.

<table>
<thead>
<tr>
<th>Study reference</th>
<th>No. of patients</th>
<th>Intervention</th>
<th>Outcome regarding CMV infection</th>
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<tr>
<td>[127]</td>
<td>50</td>
<td>IVIG</td>
<td>No significant effect</td>
</tr>
<tr>
<td>[128]</td>
<td>104</td>
<td>IVIG plus either acyclovir or sequential ganciclovir/acyclovir</td>
<td>Reduced incidence of infection and disease with IVIG/ganciclovir/acyclovir combination</td>
</tr>
<tr>
<td>[129]</td>
<td>120</td>
<td>Acyclovir or placebo</td>
<td>Reduced incidence of infection and disease in CMV-seropositive recipients</td>
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<tr>
<td>[130]</td>
<td>146</td>
<td>CMVIG or placebo</td>
<td>Reduced incidence of CMV disease in low- and moderate-risk groups with CMVIG</td>
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<tr>
<td>[131]</td>
<td>143</td>
<td>Acyclovir or sequential ganciclovir/acyclovir</td>
<td>Reduced incidence of CMV infection and disease in low- and moderate-risk groups with ganciclovir/acyclovir</td>
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<tr>
<td>[132]</td>
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<td>Preemptive ganciclovir or acyclovir</td>
<td>Reduced incidence of CMV disease with preemptive ganciclovir</td>
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<tr>
<td>[133]</td>
<td>250</td>
<td>Ganciclovir or acyclovir for 100 d</td>
<td>Reduced incidence of CMV infection and disease in all risk groups with ganciclovir</td>
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NOTE. CMVIG = CMV hyperimmune globulin; IVIG = intravenous immune globulin.
Table 2. Comparative efficacy and cost of prophylactic therapy for CMV disease in liver transplant recipients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CMVIG</th>
<th>Preemptive ganciclovir</th>
<th>Sequential ganciclovir/high-dose acyclovir</th>
<th>Prolonged ganciclovir</th>
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<td>Efficacy for:</td>
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<td>Primary disease</td>
<td></td>
<td></td>
<td>None</td>
<td>+++</td>
</tr>
<tr>
<td>Superinfection and/or reactivation</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Prevention of bacterial and fungal</td>
<td>+</td>
<td>Unknown</td>
<td>Unknown</td>
<td>+</td>
</tr>
<tr>
<td>superinfection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost (U.S. $) of monitoring for viral shedding</td>
<td>NA</td>
<td>2,000*</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Length of therapy</td>
<td>6 or 7 doses over 112 d</td>
<td>7 d</td>
<td>90 d</td>
<td>100 d</td>
</tr>
<tr>
<td>Cost (U.S. $) of therapy</td>
<td>6,000–12,000</td>
<td>N/A†</td>
<td>2,800</td>
<td>13,000</td>
</tr>
</tbody>
</table>

NOTE. CMVIG = CMV hyperimmune globulin; NA = not applicable; +, ++, and +++ denote mild, moderate, and marked efficacy, respectively. * CMV shell vial cultures of blood and urine at 2, 4, 6, 8, 12, 16, 20, and 24 weeks. t Therapy given for 7 days only upon evidence of viral shedding.

Disease [124, 125, 131]. This combination therapy was also associated with a significant delay in the onset of CMV infection (mean, 79 days) in comparison with that in patients receiving low- or high-dose acyclovir alone (means, 38 and 45 days, respectively) [125]. However, this benefit was mostly limited to low- and intermediate-risk groups; no benefit was identified for patients at risk for primary infection, i.e., the seronegative recipients of seropositive organs.

A recent randomized trial comparing ganciclovir and high-dose acyclovir and involving 250 patients was reported by Winston et al. from UCLA [131]. Patients were randomized to receive either intravenous ganciclovir (6 mg/[kg·d] during postoperative days 1 to 30 and then 6 mg/[kg·d] Monday through Friday until day 100) or acyclovir (10 mg/kg iv every 8 hours until discharge and then 800 mg orally four times a day until day 100). CMV infection occurred in 48 (38%) of 126 patients receiving acyclovir but in only 6 (5%) of 124 patients receiving ganciclovir. CMV disease developed in 12 (10%) of 126 acyclovir recipients but in only 1 (0.8%) of 124 ganciclovir recipients. The protective effect of ganciclovir was observed in high-risk patients such as those receiving OKT3 antirejection therapy and seronegative recipients of organs from seropositive donors. Few late cases of CMV infection and disease (5 and 2 patients, respectively, in the ganciclovir group) were noted to occur in both treatment arms when antiviral therapy ended, i.e., after day 120.

Combination antiviral therapy with ganciclovir and foscarnet has not been investigated in cases of solid-organ transplantation but may hold promise, because these agents are synergistic for antiviral activity in vitro [143]. Currently, no controlled data are available on foscarnet or on the combination of ganciclovir and CMVIG in preventing CMV disease in liver transplant recipients. Data from preliminary reports of CMVIG administered for 4 months in addition to a short course of ganciclovir (10 mg/kg daily for 2 weeks), with or without high-dose acyclovir for 10 weeks, appear promising [144]. This combination requires further study in terms of efficacy, cost, and potential applicability.

Preemptive therapy. Preemptive therapy is defined as highly effective therapy administered during a brief period to...
patients who are at highest risk for serious disease [145]. The advantage of preemptive therapy over broad prophylactic regimens is that it is administered only to those most likely to benefit from it, thereby avoiding unnecessary prophylaxis in patients who are not at risk for CMV-associated morbidity and death. Candidates for preemptive therapy include recipients with asymptomatic viremia and patients with latent infection at risk for reactivation (such as those receiving antilymphocyte therapy). In a study of seropositive liver transplant recipients treated with OKT3 for steroid-resistant rejection, preemptive ganciclovir therapy caused a delay in the time of onset of CMV infection and a decrease in the frequency and severity of CMV disease [146].

Singh et al. [132] recently reported from the University of Pittsburgh on a randomized trial comparing high-dose acyclovir with a short course of preemptive ganciclovir therapy to prevent CMV disease in liver transplant recipients. Patients were stratified by their CMV antibody status and the CMV antibody status of the donor and were assigned to a high-dose acyclovir group (n = 24) or an experimental group (n = 23), who received no prophylaxis. If CMV infection was documented (by surveillance shell vial cultures of blood and urine, done at 2, 4, 6, 8, 12, 16, and 24 weeks), ganciclovir (5 mg/kg twice daily) was administered for 7 days to the experimental group. Both groups had comparable rates of CMV shedding (22% and 25%). CMV disease developed in seven patients (29%) in the acyclovir group and in one patient (4%) in the experimental group (P < .05).

Two points in relation to this study are worth considering: (1) all patients received FK506-based immunosuppression, which may be associated with a lower risk of CMV infection than that associated with cyclosporine-based immunosuppression [133, 147], and (2) of all 47 patients, only 5 (10.7%) belonged to the group at high risk for CMV infection and disease post-transplantation (1 patient received OKT3 therapy, and only 2 patients in each treatment arm were seronegative recipients of a seropositive allograft). It appears, therefore, that short-course preemptive ganciclovir is a safe, attractive, and potentially cost-effective prophylactic regimen for seropositive liver transplant recipients but remains of questionable value for high-risk patients, including seronegative recipients of seropositive grafts and patients requiring antilymphocyte therapy.

The need for CMV prophylaxis has to be interpreted in light of its impact on long-term graft and patient survival, in addition to economic considerations. For a prophylactic regimen to be widely applicable across all risk groups, it must be effective, practical, safe, and inexpensive. A comparison of the benefit, toxicity, and cost of various prophylactic regimens is critical in determining the appropriate choice of therapy (table 2). Based on the above, a working algorithm for CMV prophylaxis at the time of transplantation is proposed (figure 3).

Conclusions

Following liver transplantation, CMV is a major viral pathogen that results in increased morbidity and affiliated costs. The spectrum of CMV infection in liver transplant recipients ranges from asymptomatic viremia to life-threatening multisystem disease. The major risk factors for the development of CMV infection are the pretransplantation CMV serological status of donor and recipient and the net state of immunosuppression after transplantation. Recent advances in the diagnosis of CMV infection and disease promise to provide new ways to monitor the response of such conditions to treatment and to guide the timing of preemptive therapy.

The treatment agent of choice for established CMV disease is intravenous ganciclovir, along with judicious management of immunosuppression, a strategy likely to result in improved graft and patient survival. For low- and intermediate-risk groups, proven prophylactic strategies include use of preemptive ganciclovir, CMV hyperimmune globulin, or ganciclovir followed by high-dose oral acyclovir. The prophylactic regimen of choice remains to be clearly defined for high-risk patients, although prolonged ganciclovir therapy or combination CMVIG/antiviral therapy appears to be an effective, albeit costly, mode of prevention. Future advances in diagnosis, therapy, and transplantation immunosuppression may provide additional information toward our understanding of and approach to CMV infection and disease in liver transplant recipients.

Acknowledgment

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References

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132. Singh N, Yu VL, Mieles L, Wagener MM, Miner RC, Gayowski T. High-dose acyclovir compared with short-course preemptive gan-


