EDITORIAL

Cytomegalovirus infection after liver transplantation: Current concepts and challenges

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Abstract

Cytomegalovirus (CMV) is a common viral pathogen that influences the outcome of liver transplantation. In addition to the direct effects of CMV syndrome and tissue-invasive diseases, CMV is associated with an increased predisposition to acute and chronic allograft rejection, accelerated hepatitis C recurrence, and other opportunistic infections, as well as reduced overall patient and allograft survival. Risk factors for CMV disease are often interrelated, and include CMV D+/R- serostatus, acute rejection, female gender, age, use of high-dose mycophenolate mofetil and prednisone, and the overall state of immunity. In addition to the role of CMV-specific CD4+ and CD8+ T lymphocytes, there are data to suggest that functionality of the innate immune system contributes to CMV disease pathogenesis. In one study, liver transplant recipients with a specific polymorphism in innate immune molecules known as Toll-like receptors were more likely to develop higher levels of CMV replication and clinical disease. Because of the direct and indirect adverse effects of CMV disease, its prevention, whether through antiviral prophylaxis or preemptive therapy, is an essential component in improving the outcome of liver transplantation. In the majority of transplant centers, antiviral prophylaxis is the preferred strategy over preemptive therapy for the prevention of CMV disease in CMV-seronegative recipients of liver allografts from CMV-seropositive donors (D+/R-). However, the major drawback of antiviral prophylaxis is the occurrence of delayed-onset primary CMV disease. In several prospective and retrospective studies, the incidence of delayed-onset primary CMV disease ranged from 16% to 47% of CMV D+/R- liver transplant recipients.

Current data suggests that delayed-onset CMV disease is associated with increased mortality after liver transplantation. Therefore, optimized strategies for prevention and novel drugs with unique modes of action are needed. Currently, a randomized controlled clinical trial is being performed comparing the efficacy and safety of maribavir, a novel benzimidazole riboside, and oral ganciclovir as prophylaxis against primary CMV disease in liver transplant recipients. The treatment of CMV disease consists mainly of intravenous (IV) ganciclovir, and if feasible, a reduction in the degree of immunosuppression. A recent controlled clinical trial demonstrated that valganciclovir is as effective and safe as IV ganciclovir for the treatment of CMV disease in solid organ (including liver) transplant recipients. In this article, the author reviews the current state and the future perspectives of prevention and treatment of CMV disease after liver transplantation.

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INTRODUCTION

Throughout the four decades that have elapsed since the first successful liver transplantation in 1967, cytomegalovirus (CMV) has remained the single most common viral pathogen influencing the outcome of this procedure. Infection with CMV is not only a very common complication after liver transplantation but it also contributes significantly to the morbidity and mortality, both by direct and indirect mechanisms[1,2].

CMV is a ubiquitous herpes virus that infects 60%-100% of humans[1,2], with primary CMV infection occurring most commonly during the first 2 decades of life. If immunocompetent, the infected individuals are mostly asymptomatic or may present with a benign febrile infectious mononucleosis-like illness. However, in individuals with compromised immunity, such as liver transplant recipients, clinical disease with high morbidity may develop and, in some cases, may lead to death[1,2].

Facilitated by its ability to evade the immune system, infection with CMV results in a state of latency in several host cells[1,2]. Consequently, these cellular sites of viral latency become reservoirs of reactivation during periods of stress and cytokine release, and serve as vehicles for transmission to susceptible hosts. Both these scenario are operational in liver transplant recipients, wherein the pharmacologic-induced impairment of immune response to "endogenously reactivated" or "allograft-transmitted" CMV leads to febrile and tissue-invasive diseases[1,2]. Because of the lack of a pre-existing CMV-specific immunity, CMV-seronegative recipients of liver allografts from CMV-seropositive donors (CMV D+/R-) are at the highest risk of CMV disease and its complications[3-5].

This article reviews the current concepts and challenges in the management of CMV after liver transplantation. Historical aspects of the disease are discussed to emphasize the remarkable improvements that have been achieved over the past several years. Conversely, ongoing issues of delayed-onset and drug-resistant CMV disease are discussed in detail, to highlight future perspectives in terms of CMV disease prevention and treatment.

CLINICAL IMPACT OF CMV IN LIVER TRANSPLANTATION

Direct CMV effects

The clinical illness caused by CMV commonly manifests as fever, bone marrow suppression, and organ-invasive diseases (Table 1)[1]. These direct clinical effects are traditionally classified as CMV syndrome (fever with myelosuppression) and tissue-invasive CMV disease, which most often involves the gastrointestinal tract (in the form of CMV gastritis, esophagitis, enteritis, and colitis), although virtually any organ system may be involved[6]. Infection of the liver (i.e., CMV hepatitis) is especially common in liver transplant recipients (compared to other solid organ transplant recipients), and this may manifest with symptoms indistinguishable from acute allograft rejection[7]. The availability of sensitive tests for the rapid detection of CMV in the blood may obviate the need for liver biopsy to differentiate the CMV infection from rejection. However, in many cases, a liver biopsy is needed to differentiate or to demonstrate the co-existence of CMV disease and allograft rejection.

In the absence of effective antiviral prophylaxis, the

Table 1 Direct and indirect clinical effects of CMV after solid organ transplantation

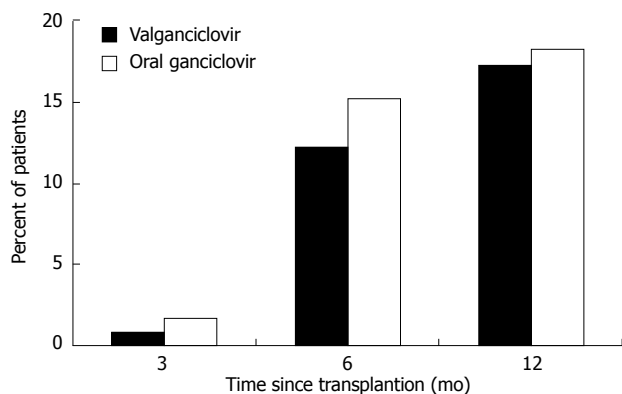| Direct effects	Indirect effects
CMV syndrome	Acute allograft rejection
Fever	
Myelosuppression	
Malaise	
Tissue-invasive CMV disease[1]	Chronic allograft rejection
Gastrointestinal disease (colitis, esophagitis, gastritis, enteritis)	Vanishing bile duct syndrome
Hepatitis	
Pneumonitis	Chronic ductopenic rejection
CNS disease	Hepatitis C virus recurrence
Retinitis	Allograft hepatitis, fibrosis and allograft failure
	Opportunistic and other infections
	Fungal superinfection
	Nocardiosis
Mortality	Bacterial superinfection
	Epstein-Barr virus and PTLD
	HHV-6 and HHV-7 infections
	Vascular thrombosis
	Mortality

[1]Any organ system may be affected by CMV.

Table 2 Estimated incidence of CMV disease during the first 12 mo after liver transplantation

	Use of anti-CMV prophylaxis	
	Yes[1]	No
CMV D+/R-	12%-30%	44%-65%
CMV D+/R+	2.7%	18.2%
CMV D-/R+	3.9%	7.9%
CMV D-/R-	0	0
All patients	4.8%	18%-29%

D: Donor; R: Recipient. [1]Most cases occur as delayed-onset CMV disease. CMV disease occurs rarely during prophylaxis with oral valganciclovir. Data adapted from[4,5,73].

direct effects of CMV occur most commonly during the first 3 mo after liver transplantation[6]. Overall, it is estimated that 18%-29% of all liver transplant recipients will develop CMV disease (Table 2)[4,5,8-10]. However, this incidence varies widely depending upon donor and recipient CMV serologic status; it may be as high as 44%-65% in CMV D+/R-, or as low as 8%-19% in CMV-seropositive liver transplant recipients (CMV R+)[4,8,10]. The incidence is markedly reduced in liver transplant recipients who receive prophylaxis with 3 mo of valganciclovir and oral ganciclovir. Recent studies have reported CMV disease rates of 12%-30% in high-risk CMV D+/R-, and < 10% in CMV R+ liver transplant recipients who received 3 mo of antiviral prophylaxis[3,4,8,10-12]. In individuals who received antiviral prophylaxis, CMV disease occurred 3 mo to 6 mo after completing antiviral prophylaxis; hence, the term "delayed-onset (also termed late-onset) CMV disease" (Figure 1)[3].

Figure 1 Time to the onset of CMV disease in solid organ transplant recipients who received 3 mo of oral ganciclovir or valganciclovir prophylaxis. Data obtained from the study by Paya et al[5].

Table 3 Selected traditional and novel factors associated with the increased risk of CMV disease after liver transplantation

Traditional factors	Recently identified factors
CMV D+/R- > CMV R+	Toll-like receptor gene polymorphism
Allograft rejection	Mannose binding lectin deficiency
High viral replication	Chemokine and cytokine defects (IL-10, MCP-1, CCR5)
Mycophenolate mofetil	Deficiency in CMV-specific CD4+ T cells
Muromonab-CD3	Deficiency in CMV-specific CD8+ T cells
Anti-thymocyte globulin	Expression of immune evasion genes
Alemtuzumab	Programmed cell death 1 expression
HHV-6	
HHV-7	
Renal insufficiency	
Others[1]	

[1]Others factors include re-transplantation, volume of blood transfusion, sepsis and factors associated with high tumor necrosis factor-α secretion[1,4,11,13,21,39-42,77,89-93].

Indirect CMV effects

The clinical impact of CMV extends beyond the direct effect of the virus. Numerous indirect outcomes, believed to be mediated by the ability of the virus to modulate the immune system have been reported (Table 1)[1,2]. CMV is known to be a potent up-regulator of alloantigens, thereby increasing the risk of acute rejection and chronic allograft dysfunction[13]. CMV is associated with vanishing bile duct syndrome and ductopenic rejection, leading to chronic cholestasis and eventually allograft failure[14-16]. Several studies have reported a higher incidence of vascular and hepatic artery thrombosis in liver transplant recipients with CMV disease, an effect that is believed to result from CMV infection of the vascular endothelial cells[17,18]. The immunomodulatory effects of CMV have also been blamed for the higher predisposition to other opportunistic infections including fungi, other viruses, and bacteria such as *Nocardia* sp.[19,20]. CMV-infected transplant recipients are more likely to develop Epstein-Barr virus-associated post transplant lymphoproliferative disorders (PTLDs), or develop co-infections with other viruses such as human herpes virus (HHV)-6 and HHV-7[19,21]. A well-described interaction between members of the beta-herpes group of viruses has been described, exemplified by the observation that reactivation of HHV-6 and HHV-7 is associated with an increased predisposition to CMV disease after liver transplantation[22-24]. In a similar manner, there is a significant association between CMV and hepatitis C virus[25-30], manifested by an accelerated course of HCV recurrence in patients who develop CMV infection after liver transplantation. In our analysis of 92 HCV-infected liver transplant recipients, there was a four-fold higher risk of allograft failure and mortality in patients with CMV infection and disease[28,30]. Three years after liver transplantation, 48% patients who developed CMV disease had allograft loss or had died, compared to 35% patients with asymptomatic CMV infection, and 17% in those who did not develop CMV infection[28,30].

Impact on mortality

CMV infection is an independent predictor of mortality

after solid organ transplantation, by mechanisms which may be direct, indirect or immunomodulatory[19,31,32]. CMV was a major cause of mortality after liver transplantation prior to the availability of intravenous (IV) and oral ganciclovir. Several recent meta-analyses have demonstrated that the use of anti-CMV drugs, either through antiviral prophylaxis or preemptive therapy, have led to significant reduction in the overall mortality after solid organ transplantation[19,33-35]. However, despite much improvement in outcome, there is emerging data to suggest that even in the contemporary era, with widespread use of antiviral prophylaxis, development of delayed onset CMV disease remains a common problem, and importantly, is associated with significantly increased risk of mortality after liver transplantation[32]. An analysis of 437 liver transplant recipients demonstrated that CMV disease occurred in 37 patients (8.5%), and its occurrence was independently associated with a 5-fold increased risk of all-cause mortality, and an 11-fold increased risk of infection-related mortality after liver transplantation[32]. The other significant and independent predictors of mortality in this study included the need for pre-liver transplant hemodialysis, a higher model for end-stage liver disease (MELD) score, and post-transplant occurrence of bacterial and fungal infections[32].

RISK FACTORS FOR CMV DISEASE AFTER LIVER TRANSPLANTATION

Lack of pre-existing CMV-specific immunity

The most common predisposing factor for the occurrence of CMV disease after liver transplantation is the lack of an effective CMV-specific immunity[4,19]. As a result, CMV D+/R- are at the highest risk of CMV disease[4,19], while CMV R+ patients have a modest risk and CMV D-/R- have the lowest risk of CMV disease after liver transplantation (Table 3).

Drug-induced immunodeficiency

The use of highly potent pharmacologic immuno-

suppression severely impairs the ability of liver transplant recipients to mount an effective immune response against reactivating CMV, thereby predisposing to increased risk of CMV disease[4,19]. The severity of immune dysfunction is particularly intense with lymphocyte-depleting drugs such as muromonab-CD3 (OKT3) and anti-thymocyte globulin[36,37]. More recently, the use of alemtuzumab has been found to be associated with higher risk of CMV disease[38]. Drugs used for maintenance immunosuppression have also been associated with CMV disease, particularly high doses of mycophenolate mofetil[30,39]. It is very likely that immunosuppressive drugs not only predispose to CMV disease, but the net state of combined pharmacologic immunosuppression increases the risk of CMV disease after liver transplantation[1,2,19].

Defects in innate and CMV-specific cell-mediated immunity

The appreciation of the role of the immune system in controlling CMV led to recent observations that inherent defects in immunity, such as mutations in the innate immunity-associated genes, increased the risk of CMV disease after liver transplantation (Table 3). In a study of 92 liver transplant recipients, a genetic polymorphism in the Toll-like receptor (TLR)-2 gene, which resulted from the substitution of arginine to glutamine at position 753 in the protein-receptor, was significantly associated with a higher degree of CMV replication and a higher incidence of CMV disease[40]. TLR2 is a pattern recognition receptor expressed in innate immune cells, and its function is to sense the glycoprotein B of CMV, thereby signaling the immune cells to produce antiviral peptides and other cytokines[40]. Our *in vitro* data suggests that this specific genetic polymorphism causes an impairment of cellular recognition of CMV by TLR2-expressing cells[40].

Likewise, the CMV-specific T cell compartment is necessary for adequate control of CMV after liver transplantation[41], although a recent study indicated that CMV-specific T cells may not necessarily predict the risk after liver transplantation[41]. There are ongoing studies in this field that may further clarify the prognostic role of CMV-specific T cell assays in stratifying CMV disease risk after liver transplantation.

Other immune measures, such as programmed death-1 expression[42] and immune evasion genes[43] have also been assessed as prognostic indicators of CMV disease after liver transplantation. In one study, programmed death-1 receptor up-regulation was significantly associated with incipient and overt CMV disease and with CMV viremia[42].

Allograft rejection

Allograft rejection *per se* is one of the most potent inducers of CMV reactivation, and thus considered a significant risk factor for CMV disease after liver transplantation[12]. Cytokines released during acute rejection, particularly tumor necrosis factor-α[44], are

potent transactivators of latent CMV[45], as demonstrated in animal models[46]. Moreover, therapy for allograft rejection with the intensification of immunosuppressive regimen further increases the risk of CMV disease both by enhancing its reactivation and by impairing the ability to generate effective cell-mediated immunity against replicating CMV[47]. Conversely, CMV induces allostimulation and increases the risk of allograft rejection, thereby creating a bidirectional relationship between CMV and allograft rejection[13].

Virus-to-virus interactions

Virus-virus interaction may influence the risk of CMV disease after liver transplantation[21,22,26-30]. Reactivation of HHV-6 has been shown to predispose to an increased incidence of CMV disease after liver transplantation[21,22,24]. In a study on 247 patients, HHV-6 seroconversion was an independent marker of CMV disease after liver transplantation. Likewise, HCV-infected liver transplant recipients also have a higher incidence of CMV disease[48], although our data in the era of valganciclovir prophylaxis has refuted this observation[25].

Degree of viral replication

The risk of CMV disease after liver transplantation is associated, in direct proportion, with the degree of CMV replication, which is partly a function of over-immunosuppression[8,23,49,50]. In one study, a viral load of 1-2860 CMV copies/10^6 peripheral blood mononuclear cells (PBMC) increased CMV disease risk by nine-fold, while viral loads $> 2860/10^6$ PBMC increased the risk by 50-fold[8].

Other factors

Other factors associated with CMV disease after transplantation include cold ischemia time, bacterial and fungal infections and sepsis, the amount of blood loss, fulminant hepatic failure as the indication for transplantation, age, female gender, Hispanic race, and renal insufficiency[2,3,19,51]. It is likely that other factors that have not yet been identified may also be influence the risk of CMV disease after liver transplantation.

PREVENTION OF CMV DISEASE AFTER LIVER TRANSPLANTATION

Because of the adverse effects of CMV on transplant outcome, its prevention is key to management of such patients[19]. Over the years, the pharmacologic agents used for CMV prevention have evolved, from the use of acyclovir[52] and immunoglobulins[53] to IV and oral ganciclovir[4] and more recently, valganciclovir[5]. There are two major strategies for CMV disease prevention after liver transplantation: (1) preemptive therapy (wherein CMV reactivation is aggressively monitored by sensitive assays and upon detection, antiviral therapy is administered preemptively to prevent its progression to clinical disease); and (2) antiviral prophylaxis (wherein

antiviral drugs such as ganciclovir and valganciclovir are administered to patients at risk of CMV disease after liver transplantation)[19]. Both strategies are highly effective in preventing CMV disease after liver transplantation[4,5,54-57]. However, antiviral prophylaxis is generally regarded as a more efficient approach and is used by the majority of transplant centers in preventing primary CMV disease in high-risk CMV D+/R- liver transplant recipients[4,8,54]. Indeed, the current American Society of Transplantation recommendation is to use antiviral prophylaxis in all CMV D+/R- liver (and other solid organ transplant) recipients[58]. Moreover, primary antiviral prophylaxis has the added benefit of reduction in bacterial and fungal opportunistic infections and mortality[33,34].

Preemptive therapy

The basic principle of preemptive therapy is to detect the presence of CMV reactivation prior to the onset of clinical symptoms, so that antiviral drugs are administered early in order to halt the progression of asymptomatic infection to full-blown clinical disease[50,54,55,57,59]. The success of this approach relies on patient compliance with CMV surveillance[60], availability of highly sensitive CMV assay that predicts the risk of disease[61], and early administration of antiviral drugs such as IV ganciclovir and oral valganciclovir[9,55,59]. With the advance in molecular diagnostic microbiology, including the availability of polymerase chain reaction (PCR), it is now possible to employ successfully preemptive therapy in liver transplant recipients (reviewed in[61]). Several studies have reported the success of IV or oral ganciclovir and valganciclovir in the preemptive treatment of CMV reactivation in liver transplant recipients, including high-risk CMV D+/R- patients[56,59]. However, some studies have indicated that preemptive therapy may not be completely effective in CMV D+/R- liver transplant recipients since the replication kinetics of CMV in immune-deficient individuals is so rapid[49] that it may result in clinical illness prior to CMV detection with once a week PCR assay[8,54]. Indeed, in our clinical experience, nearly 25% of CMV D+/R- liver transplant recipients who developed CMV disease were not identified early by a protocol-based weekly CMV PCR assay[8,54]. Accordingly, the current guideline from the AST does not recommend preemptive approach in CMV D+/R- liver transplant recipients[58]. However, this approach is recommended, and is highly effective, in CMV-seropositive liver transplant recipients. Reassuringly, clinical trials have demonstrated the efficacy of preemptive therapy in CMV disease prevention[54-56,59]. Three meta-analyses that collectively analyzed data from prospective clinical trials confirmed the efficacy and benefits of preemptive therapy in the prevention of CMV disease[34,35,62]. When conducted properly, preemptive therapy, with the use of oral ganciclovir, IV ganciclovir, or valganciclovir resulted in reduction of CMV disease by about 70%[34,35,62]. Moreover, preemptive therapy is not associated with late onset CMV disease (unlike with antiviral prophylaxis,

as discussed below)[55,59]. Currently, valganciclovir is the most commonly used drug for preemptive therapy, and in one study, was demonstrated to be as effective in terms of clinical and virologic response, when compared with IV ganciclovir[55,59]. In addition, preemptive therapy may be beneficial in reducing the indirect effects of CMV. In one study, the incidence of major opportunistic infections, bacteremia, bacterial infection, HCV recurrence, and rejection were not significantly different between liver transplant patients who received preemptive therapy and those who did not have CMV reactivation[63].

Antiviral prophylaxis

Several clinical trials have demonstrated that antiviral prophylaxis is highly effective in preventing the direct, and possibly the indirect effects of CMV after liver transplantation[4,5]. Recent meta-analyses have highlighted the clinical benefits[34,35,62]. Compared to placebo or no treatment, patients who received antiviral prophylaxis had lower incidence of CMV disease (58%-80% reduction) and CMV infection (about 40% reduction)[62]. In one meta-analysis, a 25% reduction in the incidence of acute allograft rejection was also observed[34]. In two studies, a reduction in all-cause mortality was also observed[34,62], mainly due to a decline in CMV-related death[62]. A reduction in the incidence of other herpes viruses, bacterial, and protozoal infections was also observed[62]. Indeed, a survey of several transplant centers showed a general preference for antiviral prophylaxis over preemptive therapy in the prevention of CMV disease in CMV D+/R- and R+ liver transplant recipients.

Acyclovir prophylaxis

The use of acyclovir as anti-CMV prophylaxis after liver transplantation has been supplanted by ganciclovir (and valganciclovir) because of the superior efficacy of the latter drugs in CMV disease prevention. In a study on 143 liver transplant recipients, CMV infection developed in 61% patients who received 3 mo of high-dose oral acyclovir compared to 24% patients who received 14 d of IV ganciclovir followed by 3 mo of acyclovir ($P < 0.001$)[64]. In a second study, 57% and 23% patients in the acyclovir group compared to 37% and 11% patients in the ganciclovir-acyclovir group developed CMV infection and disease, respectively[52]. In a third randomized controlled trial on 250 liver transplant recipients, CMV infection and disease occurred in 38% and 10% of patients in the acyclovir group, respectively, compared to 5% and 1% in the ganciclovir group, respectively[65].

Ganciclovir prophylaxis

The current data indicates that ganciclovir-based regimen is more effective (compared to acyclovir and immunoglobulins) in reducing the incidence of CMV after liver transplantation. In one study, the administration of IV ganciclovir for 90-100 d reduced the incidence of CMV disease in CMV D+/R- liver

transplant recipients to 5.4% (compared to 40% in patients who received < 7 wk of prophylaxis)[65]. The major drawback to IV ganciclovir was the need for long-term IV access and the risk of thrombosis, phlebitis, and line-associated infections[37,66]. Subsequently, oral ganciclovir became available, and in a landmark randomized trial that compared the drug with placebo, oral ganciclovir for 98 d reduced significantly the 6-mo incidence of CMV infection (51.5% *vs* 24.5%; *P* < 0.001), and CMV disease (19% *vs* 5%; *P* < 0.001) in liver transplant recipients[4], including CMV D+/R- patients (44% *vs* 15%, *P* = 0.02) and patients who received antilymphocyte antibodies (33% *vs* 5%; *P* = 0.002)[4]. Among CMV R+ liver transplant recipients, oral ganciclovir for 12 wk reduced the incidence of CMV disease to 1% (compared to 7% in patients who received acyclovir)[67]. These studies were in support of the United States FDA approval of oral ganciclovir prophylaxis for the prevention of CMV disease in liver transplant recipients. Oral ganciclovir, however, is poorly absorbed, and its oral administration results in low systemic ganciclovir levels[68]. This factor has been implicated in the emergence of ganciclovir-resistant CMV in certain clinical settings[69,70], such as high-risk CMV D+/R- patients, and those receiving potent immunosuppressive regimens.

Valganciclovir prophylaxis

Valganciclovir, a valine ester of ganciclovir, which results in enhanced absorption, resulting in systemic drug levels that are comparable to IV ganciclovir[68,71]. Pharmacokinetic studies indicate that a 900 mg dose of valganciclovir achieves a similar daily area under the concentration time curve (AUC_{24}) as an IV dose of 5 mg/kg of ganciclovir[68]. The role of valganciclovir in the prevention of CMV disease after liver transplantation was evaluated in a multicenter randomized non-inferiority clinical trial that compared it with oral ganciclovir in a cohort of 364 CMV D+/R- solid organ transplant (including liver) recipients (Figure 1)[5]. Overall, the 6-mo incidence of CMV disease was 12% and 15% in the valganciclovir and oral ganciclovir groups, respectively[5]. Follow-up at one year, demonstrated that the incidence of protocol-defined CMV disease in all patients was 17.2% and 18.4% with valganciclovir and oral ganciclovir, respectively[5] (Notably, the incidence of investigator-determined CMV disease cases was about 28% and 30%, respectively).

However, in 177 liver transplant recipients who participated in the clinical trial, the incidence of CMV disease was 19% in the valganciclovir group as opposed to only 12% in the ganciclovir group[5]. There was also a higher incidence of tissue-invasive CMV disease in the valganciclovir group. While the clinical trial was not designed to determine differences between the transplanted organs, these results raised skepticism about the efficacy of valganciclovir prophylaxis after liver transplantation. As a result of these findings, valganciclovir prophylaxis did not gain approval from the US-FDA for prophylaxis against CMV disease after

liver transplantation (valganciclovir received approval for prevention of CMV disease in heart, kidney, and pancreas recipients). Although not FDA-approved for prophylaxis in liver transplant recipients, valganciclovir is the most widely used drug for the prevention of CMV disease after liver transplantation[72].

The efficacy of valganciclovir (and oral ganciclovir) prophylaxis is undermined by the emergence of late-onset CMV disease (Figure 1). In a retrospective study on 203 liver transplant recipients who received valganciclovir 900 mg daily for 3 to 6 mo, the overall incidence of CMV disease was 14%[73]. The incidence varied among the different CMV serogroups (16% in D+/R+ group; 7% in D-/R+ group; and 26% in D+/R- group)[73]. These findings illustrate that the burden of delayed-onset CMV disease remains high particularly in the CMV D+/R- group[5]. In our analysis of 67 CMV D+/R- liver transplant recipients who received 3 mo of oral ganciclovir and valganciclovir prophylaxis, the two year incidence of CMV disease was 29%[3]. The incidence of delayed-onset CMV disease was not significantly different between patients who received oral ganciclovir or valganciclovir (22% *vs* 28%; *P* = 0.63)[3].

Maribavir prophylaxis (investigational)

The search for anti-CMV strategies continues to evolve with the recent entry of maribavir into clinical trials. Maribavir, a novel benzimidazole riboside compound that inhibits viral DNA assembly and egress of viral capsids[74], is now undergoing clinical trials for the prevention of primary CMV disease after liver transplantation[75,76]. Because it has a unique mechanism of action that is distinct from ganciclovir, foscarnet, and cidofovir (all of which act to inhibit CMV DNA polymerase), maribavir is expected to expand the therapeutic armamentarium against CMV[75]. So far, it does not show cross-resistance with the currently available drugs. Therefore, it has a good potential as an alternative drug for the treatment of ganciclovir-resistant CMV. In addition, maribavir provides a more favorable toxicity profile compared to foscarnet and cidofovir, both of which are highly nephrotoxic. In preliminary studies conducted in allogeneic bone marrow transplant recipients, maribavir was found to be safe and did not have myelosuppressive effects. In terms of efficacy, when compared with placebo, maribavir showed significant reduction in CMV viremia[76]. The ongoing comparative multicenter trial of maribavir and oral ganciclovir in liver transplant recipients will likely complete enrollment in 2009. In this multi-center international randomized trial, the incidence of CMV disease will be compared between patients randomized to oral maribavir, and the currently approved standard oral ganciclovir.

The challenge of delayed- and late- onset CMV disease

With the success of a 3-mo anti-CMV prophylaxis program (in terms of the almost complete elimination of CMV disease in individuals who are actively taking antiviral drugs), the challenge of delayed- and late-onset CMV disease has emerged. Indeed, in many

high-risk CMV D+/R- individuals, the use of antiviral prophylaxis has only delayed the onset of CMV disease to 3-6 mo after liver transplantation[3-5,12]. In one of these retrospective studies, CMV disease occurred in 14 of 54 (26%) CMV D+/R- liver transplant recipients who received valganciclovir for at least 3 mo[73]. Our clinical data suggests that, while no breakthrough CMV disease occurred during the 3 mo of oral ganciclovir or valganciclovir prophylaxis, 29% of CMV D+/R- liver transplant recipients developed delayed-onset primary CMV disease[3]. Thus, one out of every four CMV D+/R- liver transplant recipients will develop CMV disease after cessation of antiviral prophylaxis[3]. Delayed-onset CMV disease commonly presents as CMV syndrome, with fever and bone marrow suppression[3]. In less than half of the patients, CMV manifested as tissue-invasive disease, and frequently affected the gastrointestinal tract[3]. Factors such as age[3], female gender[3,77], renal dysfunction[77], and allograft rejection[12] predisposed to the development of delayed-onset primary CMV disease[3,12,77,78]. Delayed-onset CMV disease appears to be clinically less severe, although it is associated with significant mortality after liver transplantation[32]. Therefore, a better method for CMV prevention is needed among CMV D+/R- liver transplant recipients.

Currently, there is an ongoing effort (in kidney transplant recipients only) to assess the efficacy and safety of 3 mo vs 6 mo of valganciclovir prophylaxis. Foreshadowing what may be expected from this trial, a recent single center study on 68 CMV D+/R- kidney transplant recipients demonstrated a significantly lower incidence of CMV disease in patients who received 24 wk compared to 12 wk of oral ganciclovir prophylaxis (7% vs 31%, respectively)[79]. If this practice is proven safe and effective, it may eventually be adopted in the liver transplant field. There are concerns regarding ganciclovir resistance, drug toxicity, and cost with such a prolonged prophylactic approach. In addition, the long-term drug toxicity of ganciclovir-based regimen is not known. In animal studies, ganciclovir has been shown to be mutagenic, teratogenic, carcinogenic, and has caused aspermatogenesis, although the clinical relevance of these findings in humans is unclear[68].

Another strategy that is gaining interest is an aggressive effort to minimize immunosuppression, including the use of prednisone-free regimens. In one Kidney and Pancreas Transplant Program, the incidence of CMV disease was markedly reduced in patients receiving a steroid-free immunosuppressive regimen[80]. Many liver transplant programs (including ours) have adapted this approach, and have minimized immunosuppression gradually so that patients are maintained on tacrolimus monotherapy beyond the 4th mo after liver transplantation. In a retrospective analysis, we observed a higher incidence of CMV disease among transplant recipients who were still receiving mycophenolate mofetil and prednisone at the time they discontinue antiviral prophylaxis. The major consequence of this approach, however, is the risk of allograft rejection when the level of immunosuppression is reduced to levels lower than necessary for the prevention of allo-stimulation[13].
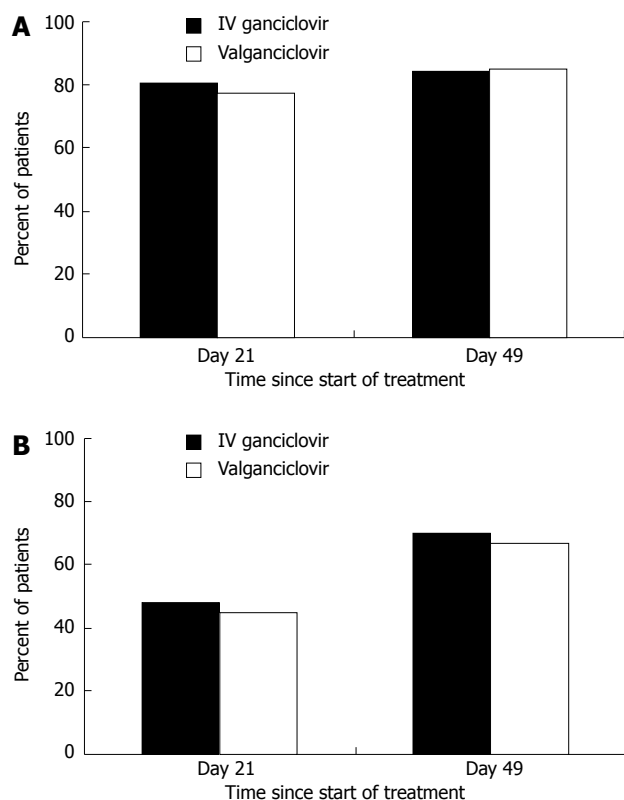
TREATMENT OF CMV DISEASE AFTER LIVER TRANSPLANTATION

The current recommendation for antiviral treatment of CMV disease after liver transplantation is IV ganciclovir[58,66,81]. Equally important is the reduction in the degree of pharmacologic immunosuppression[19]. Oral ganciclovir should not be used for the treatment of active CMV disease because of its poor bioavailability[19]. Valganciclovir, a prodrug of ganciclovir that provides high systemic ganciclovir concentrations[71], has now made it possible for oral treatment of CMV disease[68,81]. Indeed, in AIDS patients, valganciclovir is approved as induction and maintenance treatment of CMV retinitis[82]. There is good clinical data to support the use of valganciclovir for the treatment of CMV after solid organ transplantation[81]. Viral kinetic studies showed comparable viral decay between IV ganciclovir and valganciclovir[50]. In a recent study, 321 solid organ (including liver) transplant recipients with non-severe CMV disease were randomized to valganciclovir or IV ganciclovir for a fixed 21-d course, followed by valganciclovir maintenance treatment for 4 wk; the proportion of patients with viral eradication at 21 and 49 d were comparable in the IV ganciclovir and valganciclovir groups (Figure 2)[81]. The overall time to viral eradication was 21 d with valganciclovir and 19 d with IV ganciclovir[81]. The calculated viral decay was 11.5 d with valganciclovir and 10.4 d with IV ganciclovir[81]. Likewise, clinical resolution was not different between the two groups. It was noted that patients enrolled in this trial were mostly CMV-seropositive, the majority were kidney recipients, and patients with severe CMV disease were excluded. Despite these limitations, this pivotal trial now supports the use of valganciclovir for oral treatment of CMV disease, at least in selected transplant patients[81]. In many instances, valganciclovir is used as a step-down treatment when the clinical symptoms have resolved after an initial induction treatment with IV ganciclovir.

The duration of treatment of CMV disease should be individualized[58,83]. The persistence of the virus at the end of therapy (by polymerase chain reaction [PCR] or pp65 antigenemia) is associated with a higher risk of clinical relapse[84]. It is now generally accepted that multiple (at least two) weekly negative CMV PCR results should be obtained before antiviral therapy is discontinued. Although this may be true for non-tissue invasive CMV syndromes, the utility of such an approach may not necessarily apply to tissue-invasive disease, which may manifest as "compartmentalized disease"[19].

The challenge of treating compartmentalized CMV disease
Compartmentalized CMV disease refers to clinical

Figure 2 The proportion of solid organ transplant patients with resolution of clinical symptoms (**A**) and viremia eradication (**B**) at day 21 and 49 following the start of valganciclovir or IV ganciclovir treatment of CMV disease. Data obtained from the study by Asberg et al[81].

syndromes wherein the virus is detected in the affected tissues but is minimally detectable or undetectable in the blood[19]. In the current era, gastrointestinal CMV disease (in the form of gastritis, esophagitis, enteritis, colitis) constitutes the vast majority of tissue-invasive patients[3,19], and in a number of cases, this type of CMV disease is "compartmentalized." Such a clinical presentation is reminiscent of CMV retinitis, a very rare manifestation of tissue-invasive CMV disease after transplantation, that is often not accompanied by viremia[82,85]. This dilemma brings to the forefront the limitation of viral load monitoring in assessing duration of treatment. In our clinical practice, it is not uncommon to have negative blood PCR assay even when there is histologic evidence of tissue invasion. Accordingly, it has become a more common practice to perform colonoscopy or upper endoscopy to document clearance of gastrointestinal CMV disease prior to discontinuation of therapy. Our anecdotal experience however indicates that this may not be necessary in mild to moderate disease as long as sufficient therapy is provided.

The challenge of treating ganciclovir-resistant CMV disease

Ganciclovir-resistant CMV is now emerging as an important complication of prolonged antiviral drug use after transplantation[2,19,70]. Currently, ganciclovir-resistant CMV is very rarely seen in liver transplant recipients (it is more common after kidney-pancreas and

lung transplantation). Unlike lung and kidney-pancreas transplant recipients who have rates as high as 9% and 13%, respectively, the estimated incidence of ganciclovir resistant CMV after liver transplantation is < 0.5%[70,86]. Several studies have identified risk factors for ganciclovir-resistant CMV[2,19,70], including CMV D+/R- status, high levels of viral replication, potent immunosuppressive therapy, and suboptimal ganciclovir levels. The vast majority of drug-resistant cases involve the selection of viral strains with UL97 (kinase) mutation[2,19,70,75,87]. UL97 mutation generally confers resistance to ganciclovir, although in some cases, a concomitant UL54 mutation (CMV DNA polymerase) is also observed, in which case, cross-resistance with cidofovir and/or foscarnet is likely. As noted, no cross-resistance has been observed with the investigational drug, maribavir.

Drug-resistant CMV is associated with significant morbidity and mortality, and there is a very limited number of antiviral drugs (which are often toxic) available for treatment[86]. Drug-resistant CMV should be suspected when viral load or antigenemia rises or does not decline to undetectable levels despite IV ganciclovir treatment. The diagnosis is confirmed by genetic analysis to demonstrate mutational changes in UL97 and UL54 genes encoding for kinase and polymerase, respectively[70,86]. In our retrospective study of 225 CMV D+/R- solid organ transplant recipients who received 3 mo of valganciclovir prophylaxis, CMV disease occurred in 65 patients (29%), including four (8%) caused by drug-resistant CMV, judged by the failure of the viral load to decline to undetectable levels while on IV ganciclovir treatment[70,88]. In our cohort, one liver transplant recipient was clinically suspected to have ganciclovir-resistant strain, although the genotypic assay failed to document any mutations[88]. The treatment of ganciclovir-resistant CMV should be guided by genotypic analysis. In patients where foscarnet or cidofovir was used, nephrotoxicity was a major adverse effect[88]. Other potential drugs for the treatment of multi-drug resistant CMV include the off-label use of immunoglobulins and leflunomide, although data supporting their use are only anecdotal[19]. The potential clinical utility of maribavir in the treatment of resistant CMV is highly anticipated[74-76,87].

CONCLUSION

Remarkable advances in molecular diagnostics and therapeutics has led to marked reduction in the incidence and severity of CMV disease after liver transplantation, and a parallel decline in the associated morbidity and mortality. However, despite these improvements, CMV remains a common infectious complication and continues to negatively influence the outcome of liver transplantation. In addition to viral factors and pharmacologic immunosuppression, the role of innate and adaptive immune deficiencies is being recognized in the pathogenesis of CMV disease after liver transplantation. Such novel findings should provide additional avenues and opportunities for

improving our management strategies. Prevention of CMV with antiviral prophylaxis and preemptive therapy is effective, although a well-controlled trial assessing these two strategies in a head-to-head comparison is yet to be conducted after liver transplantation. Currently, valganciclovir prophylaxis is the most common approach for the prevention of CMV disease in CMV D+/R- and R+ liver transplant recipients. The availability of predictive diagnostic tests has paved the way for the successful use of preemptive therapy in preventing the progression of CMV reactivation to clinical disease even in high-risk liver transplant patients. IV ganciclovir remains the standard of treatment for established CMV disease, although valganciclovir has now been shown to be equally effective in the treatment of mild to moderate CMV diseases. The duration of treatment should be individualized, depending upon clinical and laboratory parameters such as the decline of CMV load in the blood as measured by rapid and sensitive molecular testing. In this context, it is generally recommended that treatment should be continued until all evidence of active infection, such as positive CMV viral load, has resolved. Ganciclovir-resistant CMV and compartmentalized tissue-invasive disease (most commonly with gastrointestinal CMV disease) are emerging challenges to the management of CMV after liver transplantation. These, together with the common occurrence of late-onset CMV disease in high-risk patients, should serve as catalysts to the ongoing search for the optimal preventive strategy for CMV disease after liver transplantation.

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