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Review Article

Human Cytomegalovirus Infection in Solid-Organ Transplantation

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Human cytomegalovirus (CMV) continues to be a major threat against solid-organ transplant recipients despite significant advancements in its prophylaxis and therapy. Primary CMV infection or reactivation of latent CMV in the transplant recipients may cause CMV diseases such as flu-like viral syndrome and tissue-invasive CMV disease. In addition, CMV infection in the recipients is associated with graft rejection and higher risk of other opportunistic infections, which are collectively known as the "indirect effects" of CMV infection. Prevention strategies with antiviral drugs including ganciclovir remarkably decreased CMV disease and the "indirect effects". Two commonly employed strategies are universal prophylaxis and preemptive therapy. However, gangciclovir-resistant CMV has emerged due to mutations in CMV UL97 and UL54 genes, now requiring alternative therapeutic options to be developed. This review provides an overview of CMV infection and disease, "indirect effects" on hosts, prevention strategies, and drug resistance in solid-organ transplant recipients.

Key Words: Cytomegalovirus, Organ transplantation, Immunocompromised hosts

I. INTRODUCTION

Human cytomegalovirus (CMV; human herpesvirus 5) contains a linear double-stranded DNA genome of approximately 235 kbp which codes for roughly 165 genes (1). The icosahedral nucleocapsid is enclosed by a lipid bilayer envelope which contains viral glycoproteins. CMV generally causes subclinical infections, then establishing a lifelong latent and non-productive infection in healthy individuals. Since CMV is a ubiquitous pathogen, approximately 70~100% of the world's population shows seropositivity, the evidence of infection (2).

Solid-organ transplantation is the only therapeutic option for many end-stage organ diseases. In allogeneic solid-organ transplant recipients, immunosuppressive agents should be used to prevent graft rejection. However, non-specific suppression of cell-mediated immunity allows reactivation of latent CMV and severe CMV infection in the recipients. Although antiviral drugs including ganciclovir have been widely used, CMV infection is still highly common amongst solid-organ transplant recipients, resulting in serious morbidity and occasional mortality (3). This review provides a brief overview of CMV infection and disease, indirect effects on hosts, prevention strategies, and drug resistance in solid-organ transplant recipients.

II. CMV infection and disease

CMV infection is defined as showing the evidence of viral replication in any body fluid or tissue whether or not symptoms are present. CMV disease is defined as showing

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Table 1. Definitions of cytomegalovirus (CMV) infection and disease

1. CMV infection: evidence of CMV replication (with or without symptoms)
2. CMV disease: evidence of CMV infection with symptoms
1) CMV syndrome; flu-like illness (with bone marrow suppression)
2) Tissue-invasive CMV disease; specific organ involvement, most commonly gastrointestinal tract

evidence of CMV infection accompanied by clinical symptoms (3). In healthy hosts, primary CMV infections are usually asymptomatic. However, symptomatic primary CMV infection or reactivation of an existing latent CMV may occur in immunocompromised hosts. The CMV disease is further categorized into CMV syndrome and tissue-invasive CMV disease (Table 1). CMV syndrome generally manifests as flu-like illness; it accompanies fever and malaise, which is commonly associated with thrombocytopenia or leukopenia induced by bone marrow suppression. Tissue-invasive CMV disease develops as a result of specific organ involvement. Although any organ can be affected, involvement of the gastrointestinal tract is most common, manifested by CMV gastritis, esophagitis, enteritis, and colitis (4, 5). In addition, tissue-invasive CMV disease tends to affect the transplanted allograft, provoking graft loss (6).

In solid-organ transplantation, highly potent immunosuppressive agents should be used to prevent graft rejection. However, this non-specific immunosuppression severely impairs the ability of the recipients to mount an effective immune response against pathogens including CMV. Therefore, reactivation of latent CMV occurs in a recipient who was seropositive (R^+) prior to the transplantation, thereby predisposing the recipient to an increased risk of CMV disease. In addition, symptomatic primary infection may occur when an organ from a CMV-seropositive donor is transplanted to a CMV-seronegative recipient (D^+/R^-). Since T-cell responses are crucially important for immune control of CMV infection, recipients receiving lymphocyte-depleting agents such as muromonab-CD3 (OKT3) and anti-thymocyte globulin (ATG) are predisposed to CMV infection in particular (7, 8).

III. Indirect effects on grafts and recipients

In addition to directly causing tissue-invasive disease, CMV infection in solid-organ transplantation is indirectly correlated with allograft rejection and higher predisposition to other opportunistic infections, which are collectively known as the "indirect effects" of CMV infection (4).

CMV infection in solid-organ transplant recipients is associated with a higher risk of allograft rejection. The best evidence of this association is based on studies with the antiviral agents such as ganciclovir in humans that demonstrate less graft failure amongst a wide range of solid-organ transplant recipients (9). A meta-analysis of 17 studies demonstrated the reduction rate of allograft rejection as 26% or 53%, depending on the strategies of antiviral drug administration (10). In their process, injury of CMV-infected endothelial cells is a pivotal first step in the development of allograft rejection. CMV infection leads to production of adhesion molecules, chemokines, and proinflammatory cytokines such as IL-8 in endothelial cells (11~14). In addition to these proinflammatory changes, CMV also induces wound repair processes, which drive migration and proliferation of smooth muscle cells in the affected blood vessels (15), thereby leading to vessel narrowing. These changes, in conjunction with procoagulant effects of CMV infection (16), may induce eventual vessel occlusion, and graft failure as a consequence of ischemia (9). Manifestation varies depending on which organ is transplanted: chronic allograft nephropathy in kidney transplantation (17), bronchiolitis obliterans in lung transplantation (18), hepatic artery thrombosis and vanishing bile duct syndrome in liver transplantation (19, 20), and coronary artery disease in heart

Table 2. Universal prophylaxis versus preemptive therapy

	Universal prophylaxis	Preemptive therapy
Other herpesviruses	Prevention	No prevention
Protection against "indirect effects"	Yes	Less
Drug toxicities	Higher	Lower
Incidence of late-onset CMV disease	High in D^+/R^-	Low
Cost	Drug-related cost	Laboratory cost
Development of specific immunity	(−)	(+)

transplantation (21).

Secondary infections may be provoked by preceding CMV infections in solid-organ transplant recipients, and pharmacological prophylaxis against CMV infection lowers the chance of these opportunistic secondary infections (22). CMV replication either disrupts mucosal epithelial surfaces, thereby predisposing the patient to the secondary infections, or it may cause alterations in components of the immune system. CMV can utilize several means for evading immune responses, thereby altering the function of host immune cells. For instance, CMV encodes many proteins such as IL-10 homologue and Fc receptor homologues that alter the immune milieu of the host by modulating molecules participating in immune recognition and inflammation (23, 24). These changes may increase the risk of opportunistic fungal, bacterial, and viral infections in CMV-infected solid-organ transplant recipients. Indeed, CMV infection or disease is found to be an independent risk factor for the development of invasive fungal diseases such as aspergillosis and candidiasis in liver (25), heart (26), and lung (27) transplant recipients. CMV disease is also found to be an independent risk factor for opportunistic bacterial infections including nocardiosis in solid-organ transplant recipients (28). In terms of its relation to other viral diseases, CMV-infected solid-organ transplant recipients have a risk of developing Epstein-Barr virus-related post-transplant lymphoproliferative diseases (PTLDs) (29), or a tendency to experience the reactivation of other latent beta-herpesviruses such as human herpesvirus (HHV)-6 and HHV-7 (30). In addition, there is a clear correlation between CMV reactivation and hepatitis C virus (HCV) pathogenesis, manifested by the accelerated course of HCV recurrence and higher mortality in CMV-infected liver transplant recipients (31).

IV. Prevention strategies

With the development of antiviral drugs including ganciclovir, prevention strategies against CMV have remarkably decreased CMV disease as well as the "indirect effects" of CMV infection. In solid-organ transplant recipients, two major CMV prevention strategies (universal prophylaxis and preemptive therapy) are commonly employed (3). In universal prophylaxis, antiviral medications are administered to all at-risk patients. On the other hand, preemptive therapy involves laboratory monitoring of the recipients at regular intervals to detect early viral replication, and treating those patients during the early phase of CMV infection to prevent its further progression to disease (3, 32).

In universal prophylaxis strategy, antiviral medications (usually intravenous ganciclovir or oral valganciclovir) are started during very early post-transplantation period and maintained for a pre-determined period of time (usually in the range of 3 to 6 months). In comparison to preemptive therapy (Table 2), universal prophylaxis has the advantages of the protection against other herpesviral infections and a reduced incidence of the "indirect effects" such as graft rejection and other opportunistic infections. On the other hand, the major disadvantages of this strategy are drug toxicities (mainly bone marrow suppression) and late-onset CMV disease (CMV disease that occurs after completion

of the prophylaxis). Arthurs *et al*. reported that 29% of recipients developed late-onset CMV disease at a median of 61 days after completing universal prophylaxis, and that its occurrence is associated with increased rates of allograft loss or mortality (33). Duration of universal prophylaxis may vary depending on the donor/recipient CMV serostatus and the type of transplanted organ. Recipients with D^+/R^- serostatus are at highest risk for CMV disease. In a multicenter double-blind randomized controlled trial conducted in D^+/R^- kidney allograft recipients, the incidence of late-onset CMV disease was significantly lower in the recipients who received 200 days of universal prophylaxis, when compared to the incidence in the recipients who received 100 days of prophylaxis. In this study, the cumulative incidence of CMV disease at 12 months of follow-up was reduced from 36.8% to 16.1% by the extended duration of universal prophylaxis (34). Lung transplant recipients are at much higher risk of CMV disease, when compared to other solid-organ transplant recipients. In a randomized multicenter trial conducted in lung transplant recipients, which compared the efficacy of 3 months versus 12 months of universal prophylaxis, recipients who received the extended duration of prophylaxis had significantly decreased rates of CMV disease (32% → 4%) (3, 32, 35).

In preemptive therapy strategy, quantitative laboratory assays (usually nucleic acid amplification testing) are performed at regular intervals (usually weekly) for 3 to 4 months to detect subclinical CMV replication in solid-organ transplant recipients. Once viral replication reaches a predefined threshold, antiviral medication is started to prevent its further progression to clinical disease. At that time, a treatment dose (not prophylactic dose) of intravenous ganciclovir or oral valganciclovir should be administered (3), and the antiviral medication must be maintained until the viral load falls below the threshold level. In comparison to universal prophylaxis, preemptive therapy has the advantages of lower drug-related toxicities and costs, although on the other hand higher laboratory costs occur. In addition, this strategy may allow the recipient to develop CMV-specific immunity during exposure to low-level CMV replication (36), thereby lowering the incidence of late-onset CMV disease. On the other hand, preemptive therapy does not protect against other herpesviral infections, and this strategy may not decrease the "indirect effects" of CMV infection (10). Additionally, there is a concern for rapid exacerbation of sub-threshold infection to tissue-invasive CMV disease in high risk D^+/R^- recipients, since rapidly replicating virus may be missed by regular weekly laboratory monitoring (37).

According to the meta-analysis (10) of 17 studies, both universal prophylaxis and preemptive therapy strategies are beneficial in preventing tissue-invasive CMV disease in solid-organ transplant recipients, and both strategies revealed the effectiveness in reducing allograft rejection. In a recent nationwide cohort study (38) which included 1239 solid-organ transplant recipients, both strategies effectively lowered the incidence of CMV disease. However, recipients who received universal prophylaxis had better graft failure-free survival, and universal prophylaxis may be preferred for recipients at high risk of CMV disease such as recipients with D^+/R^- CMV serostatus, lung transplant recipients (3).

V. Drug resistance

With the widespread use of universal prophylaxis and preemptive therapies, antiviral drug resistance has emerged. Although it is still uncommon (1.8~2.2%) (39, 40), the frequency of ganciclovir-resistant CMV in solid-organ transplant recipients has been increasing. Infection with ganciclovir-resistant CMV is associated with higher morbidity and mortality in solid-organ transplant recipients (41), especially in lung transplant recipients (42).

Ganciclovir is a synthetic analogue of 2'-deoxyguanosine which requires three consecutive phosphorylation steps for its antiviral activity (Fig. 1). The first phosphorylation step is carried out by a viral phosphotransferase encoded by CMV UL97 gene, yielding ganciclovir monophosphate. Cellular kinases catalyze two additional rounds of phosphorylation, yielding ganciclovir diphosphate and triphosphate, subsequently. Ganciclovir triphosphate is a competitive inhibitor of dGTP (deoxyguanosine triphosphate) at incorporation into elongating DNA, and preferentially inhibits viral DNA

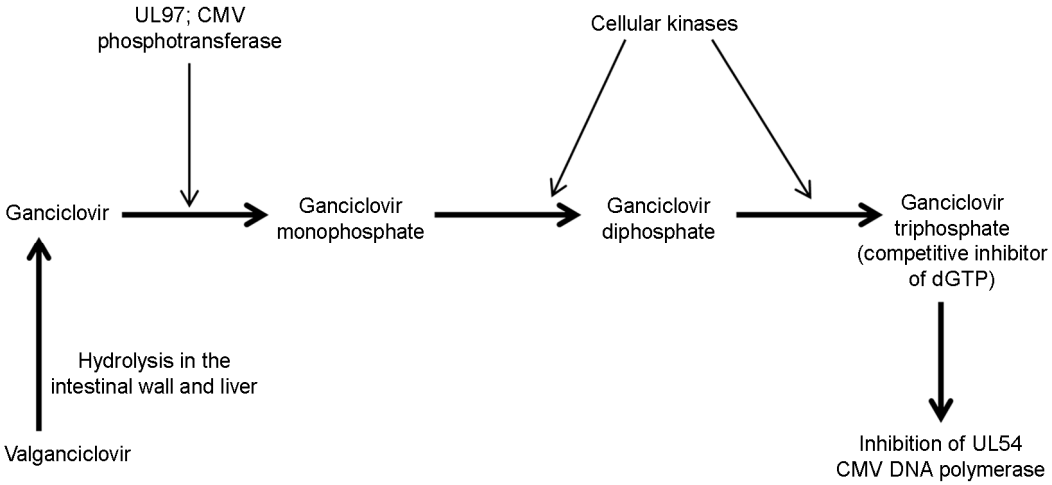

Figure 1. Ganciclovir metabolism and mode of action. Ganciclovir requires three consecutive phosphorylation steps for its antiviral activity. The first phosphorylation step is carried out by a viral phosphotransferase encoded by CMV UL97 gene. Cellular kinases catalyze two additional rounds of phosphorylation. Ganciclovir triphosphate is a competitive inhibitor of dGTP (deoxyguanosine triphosphate), and preferentially inhibits viral DNA polymerases encoded by CMV UL54 gene. Valganciclovir is a prodrug of ganciclovir.

polymerases encoded by CMV UL54 gene more than cellular DNA polymerases. Incorporation of ganciclovir triphosphate by the viral DNA polymerase alters the DNA conformation, thereby slowing the elongation and replication of viral DNA (43). Valganciclovir is a L-valyl ester prodrug of ganciclovir, and has a better bioavailability. Valganciclovir is rapidly hydrolyzed to ganciclovir in the intestinal wall and liver (44), and then converted to ganciclovir triphosphate in the same manner that ganciclovir is metabolized.

Resistance to ganciclovir in CMV is most commonly conferred by mutations in the UL97 phosphotransferase gene. Drug resistances may result from mutations that either prevent binding of ganciclovir to the UL97 phosphotransferase or alter the conserved residues for the phosphorylating activity of the phosphotransferase (45, 46). Ganciclovir resistance in CMV is less commonly conferred by the UL54 DNA polymerase gene mutations that either prevent binding of the ganciclovir triphosphate to the UL54 DNA polymerase or alter the balance of enzyme activities toward the removal of the incorporated drug (42). The most significant risk factor of ganciclovir-resistant CMV is D^+/R^- serostatus (39). Additionally, there are other risk factors such as receipt of lung transplantation, intense immunosuppressive treatment, high pre-treatment CMV viral load, and exposure to sub-therapeutic levels of ganciclovir (47). Resistance to ganciclovir should be suspected in patients showing no improvement after 6 weeks of adequate therapy especially in the presence of aforementioned risk factors (3). Genotypic assay that detect the presence of specific mutations in the UL97 gene should be performed when ganciclovir resistance is suspected. In patients treated with ganciclovir, mutations in UL54 gene usually occur after UL97 mutation, and high-level resistance to ganciclovir may be rendered by the combined UL54-UL97 mutations (42). In patients with low-level resistance to ganciclovir conferred by some UL97 mutations, escalated dose of intravenous ganciclovir may be sufficient. In patients with high-level resistance to ganciclovir, switching to intravenous foscarnet (a pyrophosphate analogue that acts directly on the viral DNA polymerase) is recommended (3, 48).

VI. Closing remarks

With the development of antiviral drugs including ganciclovir, prevention strategies reduced CMV disease and "indirect effects" of CMV infection. However, the risk of

CMV remains high and continues to be a significant threat to solid-organ transplant recipients. Besides, ganciclovir-resistant CMV has emerged. Therefore, the development of alternative therapeutic options is required. Experimental new antiviral drugs including hexadecyloxypropyl cidofovir (CMX001) are undergoing clinical trials (49). Several CMV vaccines are in early stages of clinical development, and further improvement is required to develop an efficacious vaccine (3, 50). Alternatively, a cautious reduction of immunosuppression intensity in solid-organ transplant recipients may allow the generation of CMV-specific immunity and further reduce the incidence of late-onset CMV disease. Ideally, developing an alloantigen-specific immune tolerance induction strategy may obviate the need of current non-specific immunosuppression used for preventing allograft rejection, and liberate the solid-organ transplant recipients from the detrimental effects of CMV infection.

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