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Cytomegalovirus infection/disease after hematopoietic stem cell transplantation

Takehiko Mori · Jun Kato

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Abstract Cytomegalovirus (CMV) disease has historically been a main cause of death after allogeneic hematopoietic stem cell transplantation (HSCT). Since the introduction of prophylactic or preemptive therapy against CMV, the incidence of CMV disease has been successfully reduced. However, breakthrough CMV disease, particularly CMV gastrointestinal disease, remains one of the major infectious complications. Administration of an antiviral agent, ganciclovir, is often associated with myelotoxicity in HSCT recipients, and delayed immune reconstitution against CMV. Delayed immune reconstitution is a possible cause of the increasing incidence of late (more than 3 months after transplant) CMV disease after HSCT in this era of preemptive therapy. Foscarnet and valganciclovir are the available alternatives to intravenous ganciclovir. Foscarnet is not myelotoxic and has a toxicity profile different from ganciclovir. Valganciclovir, a prodrug of ganciclovir, has a higher bioavailability than oral ganciclovir and could be of clinical use, particularly in the outpatient setting or for patients requiring long-term antiviral therapy. Recent technological developments have enabled the visualization and isolation of CMV-specific T cells. Using these techniques, an individualized approach could be conducted based on each patient's immune reconstitution against CMV. In this review, we summarize the recent progress and current knowledge of CMV infection and disease after allogeneic HSCT.

Keywords Cytomegalovirus · Hematopoietic stem cell transplantation · CMV antigenemia · Preemptive therapy · Prophylactic therapy · Real-time PCR · Ganciclovir · Foscarnet · Immunotherapy

1 Introduction

Cytomegalovirus (CMV) is still one of the major causes of infectious complications after hematopoietic stem cell transplantation (HSCT), although several new prophylactic options for CMV infection/disease have been made available [1]. Among the recipients of HSCT, CMV causes pneumonia, gastroenteritis, and less commonly retinitis and hepatitis. Because of the initial high mortality of CMV pneumonia (80–90%), transplant physicians have long sought strategies to reduce its morbidity and mortality. Recent advances in diagnostic measures such as CMV antigenemia and polymerase chain reaction (PCR) and the introduction of effective antiviral agents have enabled sufficiently early detection of CMV to suppress reactivation and prevent subsequent development of CMV diseases (preemptive therapy). However, such approaches have yet to be standardized universally [2]. In this review, we summarize the recent progress and current knowledge of CMV infection and disease after allogeneic HSCT.

2 Serostatus of recipients and donors

For the determination of risk of developing CMV infection/disease, HSCT recipients and donors should be examined for the presence of serum anti-CMV IgG before transplantation. Although recent advances have enabled

T. Mori (✉) · J. Kato
Division of Hematology, Department of Medicine,
Keio University School of Medicine, 35 Shinanomachi,
Shinjuku-ku, Tokyo 160-8582, Japan
e-mail: tmori@sc.itc.keio.ac.jp

effective prevention of CMV-related mortality, CMV seropositivity of the recipients still remains an independent risk factor for poor prognosis and mortality [3]. Recipients of T cell-depleted allografts or HLA-mismatched donors are the patients predominantly affected by CMV seropositivity. Possible explanations for the deleterious effects of CMV seropositivity include an immunomodulatory effect of CMV that leads to an increase in the risk of other infections; the drug toxicity of antiviral agents which are more frequently used in CMV-seropositive patients; or the direct effects of CMV diseases.

The CMV serostatus of the donor has also been reported to affect the outcome of the HSCT, although this association is still controversial. Ljungman et al. [4] reported the results of an analysis of 7018 CMV-seropositive HSCT recipients, which showed that HSCT from CMV-seropositive donors was associated with better survival than HSCT from CMV-seronegative donors when the donors were unrelated, but not when the donors were HLA-identical siblings. In regard to the HSCT for CMV-seronegative recipients, grafts from CMV-seropositive donors had had poorer survival [5]. Thus, the impact of donor CMV-serostatus is considered to depend on the CMV-serostatus of the recipients.

3 Prophylaxis of CMV infection/disease

3.1 Blood products and selection of graft donors

As described above, CMV-seronegative recipients should be transplanted from CMV-seronegative donors to prevent primary CMV infection, if possible [6]. At some institutes, donor CMV-serostatus is part of the selection algorithm even for CMV-seropositive recipients [6].

CMV-seronegative recipients receiving grafts from seronegative donors (R−/D−) have a low risk of developing CMV infection provided they receive prophylactic management against primary CMV infection, including the transfusion of blood products from CMV-seronegative blood donors, or leukocyte-reduced blood products [7, 8]. A recent meta-analysis has revealed that the use of blood products from CMV-seronegative donors is more effective than the use of leukocyte-reduced blood products in transfusion-associated primary CMV infection (despite the fact that the two were once considered equally effective) [9].

3.2 Immune globulin

Two randomized trials revealed that immune globulin had no effect in reducing the incidence of CMV diseases in CMV-seronegative recipients, although the incidence of CMV excretion was reduced [10, 11]. A recent meta-analysis by Raanani et al. [12] has shown that CMV infection was not significantly reduced by immune globulin or CMV hyperimmune globulin, while it was associated with an increased incidence of veno-occlusive disease. Thus, immune globulin should not be administered routinely for the prophylaxis of CMV infection.

3.3 Acyclovir and valacyclovir

The efficacy of acyclovir in preventing CMV infection after HSCT has been evaluated in several randomized trials, and the results are not conclusive [13–17]. The first small report ($n = 39$) used a standard dose of acyclovir (200 mg every 6 h, orally) and showed that the incidence of CMV disease was significantly reduced [13]. Other studies using high-dose intravenous acyclovir (500 mg/m^2, 3 times/day) have also revealed its efficacy in reducing CMV disease and improving survival within 100 days after transplantation [14, 16, 17]. Furthermore, high-dose acyclovir followed by oral acyclovir (800 mg, 4 times/day, for 6 months) has been shown to improve long-term survival [16, 17]. However, Selby et al. [15] reported that intravenous acyclovir (5 mg/kg, 3 times/day) followed by long-term oral acyclovir (800 mg, 4 times/day, for 6 months) had no effect on the incidence of CMV disease. One possible explanation for these disparate results among different doses of acyclovir is the inefficient activation via phosphorylation by the product of the CMV UL97 gene or cellular enzymes [18].

Valacyclovir, an L-valyl ester prodrug of acyclovir, has an oral bioavailability 3–5 times greater than that of acyclovir. Based on the promising results obtained for high-dose intravenous acyclovir, two studies compared the effect of valacyclovir (8000 mg/day) on the incidence of CMV infection/disease with that of acyclovir and ganciclovir, respectively [19, 20]. One large study ($n = 727$) by Ljungman et al. [19] reported that high-dose intravenous acyclovir followed by valacyclovir until week 18 after transplantation significantly reduced CMV infection or disease as compared with that followed by oral acyclovir (28 vs. 40%), leading to the reduced preemptive administration of ganciclovir or foscarnet. The other study reported that valacyclovir is as effective in preventing CMV infection as ganciclovir (5 mg/kg, 2 times/day) [20]. However, in the latter study, CMV infection was diagnosed by detecting CMV using culture methods in urine or blood, which is less sensitive than PCR or CMV antigenemia assays, and this might have affected the results. A lower dose of valacyclovir, 3000 mg/day, has also been shown to reduce CMV infection in a non-randomized trial [21].

Although high-dose acyclovir or valacyclovir has the potential to reduce CMV infection and disease, it should be

combined with a preemptive strategy guided by periodical CMV monitoring using sensitive assays. Furthermore, the cost-effectiveness of these treatments should also be examined.

3.4 Ganciclovir

The efficacy of prophylactic therapy with ganciclovir has been examined in two randomized trials. Although the dose and schedule of ganciclovir administration was different between the studies, prophylactic ganciclovir has been shown to reduce CMV infection and disease as compared with placebo [22, 23]. However, a survival benefit was not demonstrated, probably because of the severe neutropenia due to ganciclovir and occurrence of late CMV disease. Universal administration leads to the unnecessary exposure of about half of the patients to a possibly myelotoxic agent. In addition, based on the results of a study showing that efficacy of preemptive therapy guided by sensitive assays, prophylactic therapy is no longer generally acceptable or recommended [24]. The role of prophylactic ganciclovir should be further evaluated in high-risk patients [25].

4 Preemptive strategies

4.1 Preemptive therapy against CMV

Preemptive therapy consists of two parts: monitoring of the CMV reactivation and early intervention in those patients in whom a CMV reactivation is detected. Thus, the efficacy of preemptive therapy is wholly dependent on the sensitivity of the monitoring assay. In the early 1990s, sensitive and non-invasive assays were not available; standard culture methods were used, and invasive methods such as bronchoalveolar lavage fluid were used as a sample in some studies [26, 27]. Although such approaches were effective in reducing CMV diseases, they were not satisfactory and were not generally accepted. Since the introduction of CMV antigenemia and PCR into clinical practice, the efficacy of preemptive therapy guided by these sensitive assays has been assessed [24, 28]. The results of a study by Boeckh et al. [24] have shown that CMV antigenemia-guided preemptive therapy results in a significantly higher incidence of CMV disease before day 100 after transplantation than prophylactic therapy, while the incidence is not significantly different by day 180 after transplantation. No difference in survival was observed. Prophylactic therapy was associated with an increased incidence of late CMV diseases and fungal infections. A possible mechanism for the increased incidence of late CMV disease in prophylactic therapy is the delayed recovery of CMV-specific T cell responses due to efficient suppression of CMV replication by ganciclovir [29]. Thus, preemptive therapy guided by CMV antigenemia or PCR is now more widely used than prophylactic therapy as a strategy against CMV after allogeneic HSCT.

4.2 Threshold for initiating antiviral therapy in preemptive therapy

The threshold of monitoring assays for initiating preemptive therapy has not been validated and remains undetermined, since these assays utilize a wide range of techniques and samples that are not readily comparable. The most used assays are the CMV pp65 antigenemia assay and quantitative PCR (real-time PCR). The CMV antigenemia assay has disadvantages as compared with real-time PCR, including the subjectivity of the method used to count the positive cells, limitations for use in neutropenic patients, and low sensitivity in CMV gastrointestinal diseases [24, 30]. Since the introduction of preemptive therapy, most of the breakthrough CMV diseases have been CMV gastrointestinal diseases, some of which might be prevented by a more sensitive PCR [30]. In our retrospective study, only 21% of patients developed a positive CMV antigenemia before developing CMV gastrointestinal disease, while 50% yielded positive results by real-time PCR [30].

It is not recommended that a universal threshold be applied irrespective of the risk factors of the patients, because not all patients with CMV infection develop CMV disease. Thus, a lower threshold should be used for high-risk patients, and a higher threshold should be used for low-risk patients. Such a risk-adapted approach could refine the uniform preemptive therapy by reducing the patients who receive toxic antiviral agents without increasing the incidence of breakthrough CMV disease [31–34].

Even very low-risk patients, such as CMV-seronegative recipients receiving grafts and blood products from CMV-seronegative donors, should undergo CMV monitoring like other patients. Although their risk may be slight, these patients must also be considered at risk of developing CMV infection or disease [35].

4.3 Dose of ganciclovir in preemptive therapy

The dose of ganciclovir in the setting of preemptive therapy varies among the reported studies, 5 or 10 mg/kg per day. Because of its myelotoxicity, the dose of ganciclovir should be reduced, provided this can be done without increasing the incidence of breakthrough CMV disease. Two recent reports have shown a successful outcome of preemptive therapy guided by CMV antigenemia with an initial dose of ganciclovir at 5 mg/kg per day [31, 32]. In both reports, the dose of ganciclovir was increased to

10 mg/kg per day according to the viral load after initiating preemptive therapy. Therefore, although this has not been confirmed by a randomized trial, the initial dose of ganciclovir in preemptive therapy could be safely reduced by half (5 mg/kg per day), if the option were available to adjust the dose according to the subsequent viral load.

4.4 Effective antiviral agents in preemptive therapy

Ganciclovir has been used and has played an important role in preemptive therapy [26–28, 30–34]. An alternative to ganciclovir is foscarnet, which is as effective as ganciclovir. However, foscarnet is used as a second-line agent because it has not been fully accepted as a first-line agent. The toxicity profiles of these agents are different: myelotoxicity for ganciclovir, and nephrotoxicity and electrolyte abnormalities for foscarnet. Two randomized studies have shown that foscarnet is as effective as ganciclovir in preemptive therapy against CMV, although different doses were used (180 or 120 mg/kg per day) [36, 37]. A study by Reusser et al. [37] has also shown that foscarnet causes less neutropenia than ganciclovir (4 vs. 11%), but causes more electrolyte abnormalities, such as hypocalcemia, hypomagnesemia, hypokalemia, and hypophosphatemia. However, these electrolyte abnormalities are well managed by intravenous supplementations. Therefore, foscarnet should be used more actively in the preemptive therapy against CMV infection for patients with neutropenia or developing neutropenia during therapy with ganciclovir. The optimal dose of foscarnet in the setting of preemptive therapy has yet to be decided, and clinical trials will be needed to resolve this matter.

Valganciclovir is an oral L-valyl ester prodrug of ganciclovir with a significantly higher bioavailability than oral ganciclovir. Several studies have reported the promising results of preemptive therapy with oral valganciclovir [38–41]. However, pharmacokinetic analysis of oral valganciclovir in HSCT recipients showed a higher exposure for patients receiving 900 mg of oral ganciclovir twice per day than for those receiving 5 mg/kg twice per day of intravenous ganciclovir [42]. The optimal dose of oral valganciclovir for preemptive therapy should be determined, if possible, based on the body weights of individual patients, as in the case of intravenous ganciclovir.

4.5 Risk-adapted preemptive therapy guided by real-time PCR

At our institute, real-time PCR and a reduced initial dose of ganciclovir are used selectively for high-risk patients to increase the efficacy, specificity, and safety of the preemptive therapy. High-risk allogeneic HSCT recipients defined as patients developing grade II or greater acute GVHD are weekly monitored by real-time PCR using plasma. If patients yield one positive result, ganciclovir at a daily dose of 5 mg/kg is immediately started. In the case of increasing viral load after initiating ganciclovir, the dose is increased to 10 mg/kg per day. Using this approach, the occurrence of CMV disease was successfully suppressed to an incidence of 7.7% without any cases of CMV pneumonia, and neutropenia (neutrophil $<1 \times 10^9$/L) was observed in only 10% of the patients [33]. However, a small number of patients developed septicemia during neutropenia. Thus, we now use foscarnet in patients with neutropenia at a daily dose of 90 mg/kg with sufficient fluid hydration and careful monitoring of electrolytes to avoid exaggerating neutropenia and toxicity.

5 Diagnosis of CMV disease

5.1 Definition of CMV infection and disease

A set of definitions of CMV infection and disease is available elsewhere [43, 44]. In brief, CMV infection is defined as detection of CMV virus, viral proteins, or nucleic acid in any samples (e.g., plasma, serum, urine, and tissue) from a patient. In general, CMV infection is asymptomatic, which is in contrast to CMV disease, which is accompanied by clinical signs or symptoms of affected organs. Symptomatic CMV infection is defined as CMV infection accompanied by clinical symptoms such as fever with or without cytopenia, but without signs or symptoms of end-organ CMV disease. CMV disease or CMV end-organ disease is defined as detection of CMV by one or more appropriate diagnostic tests accompanied by documentation of symptoms and/or signs of affected organs. Common organs which could be affected by CMV are the lungs, gastrointestinal tract, retina, and liver, and less commonly, the central nervous system, kidney, bladder, myocardium, and pancreas. Late CMV disease is defined as CMV disease developing later than 3 months after transplantation.

5.2 Diagnosis of CMV disease

In the era of preemptive or prophylactic therapy, CMV pneumonia has become increasingly rare despite its previous status as the most frequent and life-threatening CMV disease after HSCT, and CMV gastrointestinal disease is now the most common CMV disease. However, the mortality rate for HSCT recipients who develop CMV pneumonia remains high. In contrast to patients with acquired immune deficiency syndrome, CMV retinitis is uncommon after HSCT, which usually develops as a late CMV disease.

CMV pneumonia is diagnosed based on the signs or symptoms of pulmonary disease (hypoxemia, pulmonary

infiltrates, etc.) together with detection of CMV in lung tissue or bronchoalveolar lavage (BAL) fluid. CMV gastrointestinal disease is diagnosed based on the signs (mucosal lesion on endoscopy) and/or symptoms together with detection of CMV in a sample obtained from the upper or lower gastrointestinal tracts. For the diagnosis of other CMV diseases such as hepatitis, CNS disease, and nephritis, the same criterion is applied. Diagnostic tests of tissues, BAL fluid, or cerebrospinal fluid include classical virus isolation, histopathological and immunohistochemical examination, or in situ hybridization. Since PCR is so sensitive that it could lead to over diagnosis of CMV disease by detecting dormant CMV-DNA, it is not an appropriate asset for the diagnosis of CMV disease. As the sole exception to this rule, CMV retinitis is diagnosed based on the documentation of typical lesions by ophthalmologists. Detection of CMV-DNA in aqueous humor samples might be of some help for the definitive diagnosis of CMV retinitis [45].

Except for CMV pneumonia, tests using peripheral blood such as CMV antigenemia or PCR can be of little use in the diagnosis of CMV disease, which has been evaluated in patients with CMV gastrointestinal disease [24, 30]. Only a limited number of patients (32%) develop positive CMV antigenemia before or at the onset of CMV gastrointestinal disease [30].

6 Treatment of CMV disease

Patients with symptomatic CMV infection should be treated with effective anti-CMV agents to prevent a progression to CMV disease. For CMV pneumonia, the standard recommended therapy is intravenous ganciclovir (5–10 mg/kg per day) in combination with high-dose intravenous immune globulin (0.2–0.5 mg/kg per day), which is based on the results from non-randomized trials [46–49]. Because of the non-randomized settings in these studies, the efficacy of immune globulin in the treatment of CMV pneumonia is still inconclusive [50]. However, it would be difficult to evaluate it in a future trial because the incidence of CMV pneumonia is quite low in this era of preemptive therapy. For other CMV diseases, standard therapy consists of ganciclovir (or foscarnet) alone [51]. The appropriate duration of therapy for CMV diseases, including pneumonia, has not been evaluated, but usually 2–4 weeks of induction therapy followed by maintenance therapy for several weeks is recommended and performed. The decision to cease therapy or switch to maintenance therapy should be made based on the each patient's response to therapy, and should not be decided uniformly.

7 Post-transplant immunity and immunotherapy against CMV

CMV-specific T cells are essential in the control of reactivation of CMV. The recent progress in technologies used for identifying and/or quantifying antigen-specific T cells has facilitated the visualization, quantification, and isolation of CMV-specific T cells [52, 53]. Using these techniques, several studies have reported the successful outcome of post-transplant immunotherapy against CMV without any significant side effects [54–57]. Although they seem promising, these techniques vary widely among the studies, and have yet to be standardized. Future studies are required to determine the most efficient isolation procedures and optimal T cell populations ($CD4^+$, $CD8^+$, or both), as well as the cell dose for effective adoptive immunotherapy against CMV.

Evaluation of immunity against CMV could be of help in determining the future risk of developing CMV disease, including late CMV disease [29, 53, 58]. Such an immunity evaluation could be valuable not only for determining whether adoptive immunotherapy is indicated, but also for selecting high-risk patients who would benefit from preemptive or prophylactic therapy. Such a strategy might further individualize and refine the current preemptive therapy guided by the results of CMV surveillance.

8 Conclusion

In spite of the introduction of effective antiviral agents and sensitive diagnostics, CMV still plays a crucial role in the post-transplant course of allogeneic HSCT. Preemptive therapy has become a standard approach for CMV disease prophylaxis, and has effectively reduced the incidence of CMV disease, especially CMV pneumonia. However, breakthrough CMV gastrointestinal disease still develops even under a preemptive approach, and late CMV disease is a recently recognized disease entity. The widely used agent ganciclovir is associated with myelotoxicity, particularly neutropenia, which could contribute to a fatal bacterial or fungal infection. To avoid this toxicity, foscarnet is an option, and its efficacy, safety, and optimal dose should be more widely evaluated in allogeneic HSCT recipients mostly taking nephrotoxic agents such as calcineurin inhibitors. In addition, it is essential to further specify the high-risk patients who need strict monitoring and preemptive therapy based on a full review of the medical background, including the CMV serostatus of recipient and donor, development of GVHD, administration of systemic steroids, anti-thymocyte globulin, and alemtuzumab, and T-cell-depleted or $CD34^+$ cell-selected grafts. In the near

future, evaluation of the respective patients' immunity against CMV could be a useful asset in preemptive or prophylactic therapy in combination with such risk factors.

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