Cytomegalovirus and Lung Transplantation

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Published online: 16 June 2013
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Abstract Lung transplantation has greatly improved management of patients with end-stage respiratory failure. The main cause of long-term morbidity and mortality is chronic rejection as manifested by bronchiolitis obliterans syndrome (BOS), which occurs in 49 % of recipients within five years of lung transplantation. Cytomegalovirus (CMV) remains the most serious opportunistic pathogen for lung transplant recipients. Although prophylaxis with antiviral drugs has much reduced CMV-associated morbidity and mortality, subclinical reactivation syndromes still affect long-term outcomes. The focus on CMV has now shifted from its direct effects, for example CMV pneumonitis, to the indirect effects, including BOS, which are currently the main obstacle to long-term improvement of lung transplantation outcomes. This review will summarize the latest evidence on the diagnosis, immunology, prophylaxis, and treatment of this commonly encountered herpes virus.

Keywords Cytomegalovirus · Lung transplantation · Herpes virus · Immunosuppression · Prophylaxis · Polymerase chain reaction · Ganciclovir · Valganciclovir · Vaccine · Immune monitoring

Introduction

Since the first successful lung transplant 30 years ago, lung transplantation has become an established treatment for advanced lung and pulmonary vascular disease. An important early realization was that the lung allograft is at high risk of infection—especially viral infection, and in particular cytomegalovirus (CMV) [1, 2].

CMV is associated with a number of clinical syndromes seen in lung transplant recipients. These syndromes are usually within the lung, and obvious acute effects include pneumonitis. More importantly, indirect long-term consequences include chronic rejection (synonymous with bronchiolitis obliterans syndrome, BOS) [2, 3, 4].

Lung transplant researchers have made great efforts to find management strategies for CMV infection and BOS. Novel studies of lung transplant CMV diagnostics, mechanistic inflammatory pathways and therapeutics are bringing about great changes to lung transplantation (and, indirectly, to all solid-organ transplantation). The purpose of this review is to update the lung transplant clinician regarding these promising advances.

History

In the 1980s and early 90s, before the availability of potent antivirals, CMV infection was observed in 54–92 % of lung transplant patients [5]. Drawing from experience of other solid-organ transplants, it was recognized that donor and recipient CMV serostatus best predicted the probability of serious post-transplant infection [6]. At that time, some lung transplant units suggested that the combination of seropositive donor and seronegative recipient led to such a high risk of CMV infection that CMV matching at the time of transplant was essential [7]. More recently, with the availability of potent antivirals, this has become much less important [1, 2, 8].

Historically, diagnosis of CMV infection was on the basis of viral culture, cytopathology, or pathology evidence [5, 7]. During the 90s, CMV pp65 antigenemia was also used to monitor at-risk patients. Over the last decade, CMV diagnosis has been on the basis of quantitative polymerase chain reaction (PCR) viral load assay of plasma and, more recently, bronchoalveolar lavage (BAL) fluid [9–11]. These

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new sensitive and specific tests mean clinicians must decide what constitutes a clinically significant CMV load and how to respond to asymptomatic low-level-load cases [9–12].

Prophylactic and therapeutic strategies have been based on minimizing immunosuppression (where possible) and on use of CMV hyper-immune gamma globulin and ganciclovir [5, 7, 12]. Use of ganciclovir, particularly oral ganciclovir and, more recently, oral valganciclovir for prophylaxis and even for primary treatment, has proved particularly efficacious for CMV management in lung transplantation [2, 8, 13]. However, the optimum duration of therapy is still an active area of research two decades later [2, 13–15].

Over the last few years, studies have investigated the relationship between CMV disease and specific immunity [16–18] with the objective of targeting prophylaxis at “at-risk” patients, rather than subjecting all transplant recipients to the risks of antiviral therapy [2, 11, 13]. With widespread use of ganciclovir in the 90s, it became evident that CMV resistance was a concern [19, 20]; fortunately, resistance to antivirals has not yet proved a major problem [2, 13].

Diagnosis of CMV Infection and Disease

Consensus definitions are important to understanding and investigating this virus [13]. *CMV infection* refers to any situation, irrespective of the presence of symptoms, in which there is evidence of CMV replication. *CMV disease* indicates CMV infection with attributable symptoms. CMV infection can be:

1. primary: a new infection acquired by a CMV-naïve seronegative recipient, typically at the time of transplant;
2. reactivation: latent-CMV reactivation in a CMV seropositive recipient; or
3. re-infection: infection of a seropositive recipient with a new CMV strain [13].

Clinical syndromes associated with CMV will be discussed in more detail below, and range from asymptomatic low-level PCR positivity to clinically evident, florid viral pneumonitis [4, 15]. It is recognized that the overall viral burden or load is a key mechanistic and diagnostic indicator of current CMV disease, and possibly also of its future progression [9, 10, 15, 21]. Westall and coworkers revealed that high levels (above a threshold of 46,000 copies mL⁻¹) of CMV DNA in BAL are a better predictor of the presence of histology-proved CMV infection than is detection of CMV DNA in plasma (sensitivity and specificity of 100 % and 95 % vs. 71 % and 94 %) [15]. Riise et al. [22] described the BAL threshold value as a viral load two standard deviations above the mean for those without pneumonitis (which as such may vary across different laboratories and patient populations). Chemaly and coworkers noted

a median CMV viral load of 46,678 copies mL⁻¹ for BAL samples from patients with positive, atypical immunohistochemistry staining for CMV in lung tissue, and 1,548,827 copies mL⁻¹ for BAL samples with positive, typical immunohistochemistry staining ($p < 0.001$ compared with those without pneumonitis) [21].

A randomized study of solid-organ transplant recipients (including those receiving lung transplants) compared blood CMV DNAemia with pp65 antigenemia [23]. Antiviral treatment was instituted when levels greater than 300,000 DNA copies mL⁻¹ blood or 100 pp65-positive leukocytes were reached. The study concluded that, compared with antigenemia, a single DNAemia cutoff significantly reduced the number of patients requiring treatment and could be safely used to guide preemptive therapy for CMV infections.

A 2009 international survey of CMV management practices for lung transplantation [11] reported that 85 % of surveyed lung transplant units based CMV monitoring on a plasma CMV viral load assay (either PCR or quantitative nucleic acid amplification test), with most defining threshold values for disease. The pp65 antigenemia assay was only used by 18.6 % of units. Despite the evidence cited above, the survey reports 25.4 % of centers continuing to perform viral culture on BAL and only 13.6 % of centers measuring CMV PCR in BAL.

Our own center favors surveillance monitoring for CMV in the lung allograft at the time of routine bronchoscopy, and the procedure followed for “at-risk” patients is:

- surveillance bronchoscopy, with transbronchial biopsy (CMV inclusion bodies) and BAL (quantitative PCR for CMV DNA), at 6, 9, 12 and 18 months post-transplant; and
- plasma CMV PCR at 6, 7, 8, 9, 10, 11, 12, 15 and 18 months post-transplant; or
- testing if clinically indicated.

We rarely observe breakthrough CMV reactivation during the six-month period of valganciclovir antiviral prophylaxis and therefore do not routinely monitor for CMV during this period [24•].

Clinical Syndromes and Associations

The long-term consequences of CMV infection can be divided into direct effects of the clinical illness and end-organ injury, and indirect effects leading to long-term morbidity and mortality. As already mentioned, CMV infection (viral replication) and CMV disease (viral replication plus clinical features) are not synonymous. It is important to note that not all patients with infection develop disease. As such, the clinical syndromes of CMV range from asymptomatic viremia to CMV viral syndrome or tissue-invasive disease (e.g. pneumonitis).

CMV viral syndrome describes a flu or mononucleosis-like clinical disease without end-organ involvement [25], characterized by systemic symptoms including fevers, malaise, myalgias, and arthralgias. Thrombocytopenia and leucopenia can also feature. Tissue-invasive disease in lung transplant recipients most commonly presents as pneumonitis, associated with low-grade fever, shortness of breath, and a non-productive cough. Histologically, it is characterized by classic inclusion bodies on lung biopsy. Hepatitis, gastroenteritis, and colitis can also occur.

The indirect effects of CMV have been postulated to be associated with prolonged low-level viral replication and the resultant immune response. CMV has been associated with both acute and chronic rejection and with opportunistic infection [26, 27].

The association between CMV infection and acute cellular rejection (ACR) is reported to be mutual, with acute rejection increasing the risk of CMV and viral replication increasing the risk of acute rejection [28]. The mechanism responsible is believed to be activation of vascular endothelium and inflammatory cells and subsequent induction of proinflammatory cytokines, chemokines, and growth factors, which occurs in both CMV infection and acute rejection [29–31]. This process is believed to affect both development of ACR in the setting of CMV infection and reactivation of CMV in the setting of ACR. Additionally, augmentation of immunosuppression to treat ACR can lead to further amplification of CMV viral replication, with evidence suggesting that the risk of CMV infection more than doubles after administration of antilymphocyte agents [32]. Evidence from both renal and lung transplant recipients suggests that antiviral prophylaxis reduces cellular rejection [24•, 33, 34, 35•].

There is an emerging consensus that CMV disease is associated with development of BOS after lung transplantation [3•, 26, 29]. Snyder and coworkers reported that treated CMV pneumonitis was a risk factor for development of BOS and for reduced survival after lung transplantation [3•], and evidence of low-level viral replication (detected in BAL) within the allograft, in the absence of CMV disease, has been associated with subsequent allograft dysfunction [24•]. These analyses support other studies that have reported reduced incidence of BOS with the use of CMV prophylaxis [5, 36–38].

Immunological Monitoring for CMV

Use of immunosuppressive medication that specifically targets T-cell function is a major contributor to CMV reactivation after lung transplantation. Despite the potentially high associated morbidity and mortality from CMV disease for this patient group, studies on the precise function

of CMV-specific T-cells after lung transplantation are limited [18, 39, 40].

The CMV-naïve recipient receiving a lung allograft from a CMV seropositive donor is at highest risk of CMV-related complications because of the absence of primed CMV-specific immune responses. These patients often have CMV infection, particularly after cessation of routine antiviral prophylaxis, suggesting that at this time they may not yet have acquired protective immunity against CMV [24•]. The ability to measure and time the de-novo acquisition of host CMV-specific CD8⁺ T-cell immunity may provide insights into why CMV reactivation is so commonly observed in these “high-risk” lung transplant recipients (LTR). CMV seropositive LTR are also at risk of CMV reactivation and/or disease. T-cell-targeted immunosuppression that controls alloreactivity also impairs host antiviral immunity, potentially resulting in CMV reactivation from the latent state. Measuring longitudinal changes to host CMV-specific immunity from the time of transplant may provide insights into why some, but not all, CMV seropositive LTR experience episodes of CMV reactivation and/or disease.

Several assays can be used to measure CMV-specific T cell responses. The QuantiFERON-CMV assay (Cellestis, Melbourne, Australia) is an in-vitro diagnostic test that uses HLA-restricted CMV epitopes to stimulate CD8⁺ T-cells in whole blood [6]. Detection of interferon- γ (IFN- γ) by use of enzyme-linked immunosorbent assay (ELISA) identifies patients with CMV-specific CD8⁺ T-cells.

Immune monitoring of CMV-specific T-cell responses may reveal individuals at increased risk of CMV disease after lung transplantation, and may therefore be useful in guiding CMV prophylaxis. The QuantiFERON-CMV assay is commercially available, and in a number of clinical studies had predictive value for CMV disease [18, 41]. In particular, the presence of detectable CMV immunity may reveal patients for whom antiviral prophylaxis may be safely stopped and, conversely, the absence of CMV-specific immunity at the time that antiviral prophylaxis is usually ceased may suggest that antiviral prophylaxis should be continued until CMV-specific immunity has been established. This hypothesis is currently being evaluated by a study in which, after a fixed period of antiviral prophylaxis, patients will be randomized to either standard of care (cessation of antiviral prophylaxis) or to continued antiviral prophylaxis if the need for it is determined by use of the QuantiFERON-CMV assay. Those with a negative result will receive up to an additional six months of antiviral prophylaxis. The primary end-point of the study will be the incidence of CMV reactivation in the lung allograft within seven months of ceasing antiviral prophylaxis. The results of this study will help identify whether immunological monitoring for CMV can help reduce CMV reactivation after lung transplantation.

Hypogammaglobulinemia is a risk factor for late CMV disease (and other infections) [42], and our unit therefore requests a measure of total IgG for all patients at three and 12 months post-lung transplant. Patients with documented hypogammaglobulinemia should be considered for standard IVIg replacement therapy.

CMV Vaccines

The high incidence of CMV reactivation despite routine use of antiviral prophylaxis emphasizes the need for better strategies to control this herpes virus. Several CMV vaccines are undergoing clinical evaluation. A CMV glycoprotein B recombinant vaccine has been shown to induce neutralizing antibodies [43] and to reduce days of viremia in a study involving kidney and liver transplant recipients [44]. A vaccine containing plasmids encoding glycoprotein B and phosphoprotein 65 reduced days of viremia and time to viremia for patients undergoing hemopoietic stem-cell transplantation [45].

CMV Prophylaxis

The use of antiviral prophylaxis after lung transplantation significantly reduces the incidence of CMV pneumonitis in the lung allograft, and may also reduce the incidence of BOS [37]. There have been no large randomized trials comparing prophylactic to pre-emptive antiviral strategies for reducing CMV disease-associated outcomes for lung transplantation. Most centers advocate prophylaxis over a pre-emptive approach (whereby antiviral therapy is not routinely prescribed to at-risk lung transplant recipients but rather only started on receipt of a positive CMV isolate) [11]. Prophylactic strategies have the potential added advantage of reducing the indirect effects, including acute rejection, of CMV on the lung allograft [24•, 31]. Although prophylactic antiviral therapy reduces the incidence of CMV reactivation and disease [46, 47], cessation of therapy is often associated with CMV reactivation [29], suggesting that prolonged prophylactic strategies may be beneficial [48]. For CMV mismatches (donor CMV seropositive, recipient CMV seronegative) 6–12 months of antiviral prophylaxis is recommended [49]. Shorter (three month) courses of prophylaxis are associated with increased CMV infection and disease [35••], and even after six months of prophylaxis over 40 % of CMV-mismatched lung transplant recipients will develop CMV infection and/or disease. Likewise, a minimum of six months' prophylaxis is advised for lung transplant recipients who are CMV seropositive at the time of transplant. Weighed against this is the risk of developing CMV resistance, particularly if there is CMV

reactivation whilst on ganciclovir prophylaxis, and the added cost of either prolonged or alternative antiviral therapy [50–52]. Early post-transplant prophylactic strategies initially involve intravenous ganciclovir (5 mg kg⁻¹ twice daily), with conversion to oral valganciclovir (900 mg day⁻¹, dose adjusted for renal function) thereafter. CMV seropositive recipients receiving CMV seropositive donor lungs are at increased risk of late CMV disease compared with those receiving CMV seronegative lungs, and may benefit from prolonged antiviral prophylaxis [53]. CMV immunoglobulin (CMV Ig) is used by many centers, although the evidence for this approach is not strong [54].

CMV Treatment

There are different approaches to and thresholds for treating CMV in lung transplant recipients, ranging from only initiating therapy when CMV reactivation is associated with end-organ dysfunction to treating asymptomatic patients who have low-level subclinical CMV reactivation. Because of data revealing that any level of CMV reactivation within the lung allograft is associated with BOS, our own transplant center favors the latter approach [24•]. The treatment we prescribe is based upon the measured CMV load within the lung allograft. Severe CMV Infection is defined as one or more of:

- BAL CMV PCR >5,000 copies mL⁻¹;
- Tissue invasion: CMV inclusion bodies in transbronchial biopsy, colon biopsy, etc;
- Clinical suspicion of end-organ damage: CMV DNAemia with either abnormal LFTs, diarrhea or unexplained bone marrow suppression; or
- CMV syndrome: fever, malaise, leukopenia, and thrombocytopenia.

Treatment for severe CMV infection is at least two weeks' IV ganciclovir, followed by three months' secondary prophylaxis with valganciclovir (450 mg bd). At the treating physician's discretion, CMV hyperimmune globulin is used if believed clinically indicated.

Subclinical CMV is defined as an asymptomatic patient with CMV PCR 600–5,000 in either plasma or BAL. Treatment is valganciclovir 900 mg bd for two weeks, with no secondary prophylaxis.

Although the bioavailability of valganciclovir is excellent and it has efficacy equivalent to that of intravenous ganciclovir for treatment of CMV disease in solid-organ transplantation [36], we continue to use intravenous ganciclovir, particularly for patients with cystic fibrosis for whom systemic absorption may be impaired. Our procedure is based on measuring CMV in the lung allograft, but we also collect a contemporaneous blood sample to guide duration of

therapy. Standard therapy is for two weeks, but is extended if CMV DNA is still detectable in the blood or if symptoms persist (especially if CMV colitis is suspected). After treatment we maintain secondary prophylaxis at a lower dose of valganciclovir (900 mg od) for a further three months, on the basis that host anti-CMV immunity is at this point not sufficiently established to guarantee CMV quiescence in the absence of valganciclovir. Neutropenia is seen in up to 10 % of patients receiving valganciclovir. To minimize CMV resistance, other marrow-suppressing medications (azathioprine, mycophenolate mofetil, co-trimoxazole) should be reduced before reduction of the valganciclovir dose.

Ganciclovir Resistance

Risk factors for ganciclovir resistance include: sub-optimum ganciclovir levels, prolonged therapy, and impaired host anti-CMV immunity (CMV mismatched patients) [55]. Incidence of ganciclovir resistance after solid-organ transplantation is highest for lung transplantation and, although it may manifest as asymptomatic low-level CMV reactivation, it is more often associated with significant morbidity and mortality [56]. Ganciclovir resistance is suspected when levels of CMV viremia persist despite prolonged antiviral therapy (duration 2–6 weeks). If suspected, confirmation is via genotype analysis of CMV isolates, looking primarily for the UL97 kinase (seen in 90 % of cases) and UL54 DNA polymerase genes that induce resistance mutations for the routinely used ganciclovir and valganciclovir. Second-line therapy includes substitution by or addition of foscarnet. Alternatively, high-dose IV ganciclovir [57] and mTor-inhibitors (everolimus, sirolimus) have some in-vitro anti-CMV effects [58], and for cases of CMV resistance many centers will reduce baseline immunosuppression and convert to an mTor-inhibitor-based regimen. Newer oral anti-CMV drugs include maribavir [59] and letermovir [60].

Conclusions

CMV remains the most serious opportunistic post-lung-transplant pathogen. It is associated with chronic lung allograft dysfunction as manifested by BOS, which is currently the main obstacle to long-term survival after transplantation. Five years post-transplant almost 50 % of patients have developed BOS, leading to reduced quality of life and a median survival of only three years. The objective of antiviral prophylaxis is not only to prevent CMV disease but also to minimize CMV subclinical replication and thus limit its “indirect” effects on allograft function. Risk of CMV is individual to each patient and is related to several factors, including previous CMV exposure, level

of immunosuppression and host immunity. The optimum duration of antiviral prophylaxis is currently unknown. Although longer courses of valganciclovir reduce CMV reactivation in the first year post-transplant, late CMV reactivation after cessation of antiviral prophylaxis persists. The use of CMV immune monitoring may enable better-customized antiviral prophylaxis which is continued until CMV-specific T-cell immunity has been established, thereby reducing the incidence of late CMV and, potentially, of chronic lung allograft dysfunction.

Compliance with Ethics Guidelines

Conflict of Interest G.P. Westall has served on speakers’ bureaus for Roche.

M.A. Paraskeva declares no potential conflict of interest.

G.I. Snell has served on speakers’ bureaus for Roche.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- Of importance
- Of major importance

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