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Assessing the Full Impact of the Indirect Effects of Cytomegalovirus Following Solid Organ Transplantation

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Abstract

Human cytomegalovirus remains the leading infectious complication following solid organ transplantation and leads to a range of direct and indirect effects that contribute to patient morbidity. The primary objective of this review is to provide an up-to-date evaluation of the evidence base for the indirect effects of human cytomegalovirus in solid organ transplant recipients. We shall review data from the rat cytomegalovirus model of transplantation and also data from individual studies in human transplantation and controlled and non-controlled antiviral intervention trials to argue that the burden of evidence supports a central role for human cytomegalovirus in the indirect effects. In addition, we will review, where data is available, the likely biological mechanisms that underlie the indirect effects after transplantation. **(Trends in Transplant. 2009;1:41-52)**

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Key words

Cytomegalovirus disease. Pathology. Rejection. Graft function. Viral replication.

Introduction

Human cytomegalovirus (HCMV) infection is endemic worldwide, with rates of HCMV seropositivity ranging from 50-60% in adult populations in the developed world and up to 95% in developing nations[1-3]. The virus is a member of Herpesviridae (family *betaherpesvirinae*), with a large and complex genome with the ability to encode at least 167 unique proteins within a genome of approximately 230 kbp[4,5]. In recent years, a number of clinical strains of virus have been fully sequenced[5,6], and this is contributing to our improved understanding of the pathogenesis in both the immunocompetent and the immunocompromised host. Consistent with other members of the Herpesviridae family, HCMV establishes latency following primary infection. Although the molecular aspects of latency and reactivation have not been fully elucidated, latency is maintained in cells of the myeloid lineage, with the viral DNA being present in an extrachromosomal circular form[7]. *In vitro*, reactivation and triggering of the immediate early promoter is accompanied by chromatin remodeling and activation of histone deacetylase[8]. However, *in vivo*, very little data in the human is available, although inflammatory cytokines including tumor necrosis factor alpha (TNFα) ap-

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pear to be important especially following organ transplantation[9]. In the immunocompetent host, primary infection with HCMV is either asymptomatic or can cause infectious mononucleosis-like symptoms. However, in the immunocompromised host, the virus is able to cause a variety of diseases and contributes to morbidity and in some cases mortality.

Cell-mediated immunity plays a critical role in the control of HCMV replication as evidenced in murine model studies[10,11] and in the human system, where the adoptive transfer of HCMV-specific $CD8^+$ T-cell clones provided protection against and control of viral replication[12-15]. Furthermore, increased rates of HCMV disease have been seen in individuals with impaired T-cell immunity as a consequence of HIV infection or due to the profound immunosuppression in organ transplantation (reviewed[16]). Recent data from our laboratory and others have shown that the quality of the T-cell immune response is key in minimizing high-level HCMV replication following both renal and liver transplantation[17-19]. For example, Mattes, et al. have shown that the proportion of HCMV-specific T-cells that can secrete interferon gamma (IFNγ) following peptide stimulation is directly related to the appearance and level of HCMV DNAemia. A reduced functional capacity of HCMV-specific $CD8^+$ T-cells has also been shown by Crough, et al. in a small cohort of solid organ recipients with symptomatic CMV infection[20]. In addition, the early appearance of polyfunctional $CD4^+$ T-cells is also important in the control of replication following liver transplantation[21].

Human cytomegalovirus infection generally occurs in the first three months following solid organ transplantation, but can be delayed in patients receiving HCMV prophylaxis[22]. The risk of developing symptomatic HCMV disease is highest in heart-lung transplant (39-41%) and lowest in the kidney and liver transplant recipients (8-32%)[23]. The risk of infection and disease in solid organ transplantation depends on the pretransplant HCMV donor/recipient (D/R) serostatus, with the highest risk occurring with a positive donor into a seronegative recipient (D^+/R^-), and an intermediate risk when both donor and recipient are seropositive (D^+/R^+) or only the recipient is seropositive (D^-/R^+). In the first instance, the recipient is at risk of primary HCMV infection, and in the second scenario, the recipient can reactivate their own latent virus or be reinfected by the donor HCMV strain. The D^+/R^- serostatus is associated with a relative risk of infection more than twenty times higher than the D^-/R^- combination[22]. Our group showed in a multivariate logistic analysis that the risk of HCMV disease, in a solid organ transplant setting, associated with donor-positive serostatus was negated once controlling for HCMV load, indicating that the association of D/R serostatus was explained by elevated virus load[24,25]. In fact, HCMV load is quantitatively different in those with (seropositive recipient) or without (seronegative recipients) immunity, with the maximum viral load attained during active infection greatest in patients who are immunologically naive. Moreover, preexisting immunity against HCMV reduces the replication rate, thus increasing the virus doubling time, and the basic reproductive number, thus reducing the number of newly infected cells arising from a single infected cell[26]. The relationship between the probability of disease and the log viral load is best modeled by a sigmoid curve where, above a critical threshold, small increases in viral load result in a significantly higher probability of disease[24,27]. The aim of the antiviral therapy is therefore to either maintain the viral load at very low levels by prophylaxis, or to intervene (preemptive therapy) as soon as possible once the HCMV has been detected.

Clinical effects of human cytomegalovirus after organ transplantation

The consequences of HCMV infection in solid organ transplants have been divided into the direct and indirect effects (Fig. 1). While the direct effects have been shown to be linked to the degree of virus replication, the indirect effects seem to result from the impact of the virus on other host cell responses, including cytokine networks and the host's immune response in the setting of low level of replication, which might or might not be detectable in blood. A common feature of the direct effects of HCMV infection is the presence of fever associated with general malaise, myalgia, or arthralgia. Studies have shown that HCMV disease initially localizes within the transplanted organ, causing, for example, pneumonitis in lung transplant recipients or hepatitis in liver recipients, and subsequently spreads systemically to other organs[28]. Under these conditions, it is assumed that direct viral

replication in the target organ leads to tissue damage, although a role for immune-mediated pathology has also been implicated[29]. Nevertheless, using sophisticated mathematical modeling approaches, cumulative viral load exposure in addition to peaks in viral load are both important in the pathogenesis of many of the direct effects[30]. At the dawn of the 21st Century, the direct effects of HCMV can be minimized and in some cases eradicated through the deployment of antiviral chemotherapy, either in a preemptive mode where initiation is based on markers of viral replication such as viral load, or in a prophylactic mode. Meta-analyses have indicated that both approaches reduce the risk of HCMV disease, with odds ratios of 0.28 for preemptive trials and 0.20 for prophylactic trials[31]. The pros and cons of both of these approaches and their cost effectiveness have been discussed extensively[32-34] and, recently, two prospective studies have provided further data highlighting the advantages and disadvantages of each therapeutic modality[35,36]. At the time of writing, ganciclovir and its prodrug valganciclovir remain the agents of choice for treatment, preemptive therapy, and prophylaxis of HCMV infection following solid organ transplantation, but other drugs are undergoing development, and maribavir, an inhibitor of the HCMV UL97 protein kinase, is currently undergoing phase III studies in solid organ recipients[37].

In contrast to the direct effects of HCMV, it has been recognized for more than 20 years that HCMV is also associated with a range of other clinical conditions, which were termed the "indirect effects" by Dr Robert Rubin. The indirect effects of HCMV infection in solid organ transplant recipients include acute and chronic graft rejections (*bronchiolitis obliterans* in lung transplant recipients, vanishing bile duct syndrome in liver transplantation, and transplant vascular sclerosis), cardiovascular disease, posttransplant diabetes, and a general nonspecific immunosuppressive syndrome that leads to an increased risk of superinfection by other opportunistic infections[22,28] and posttransplant lymphoproliferative disorder (PTLD). A few case reports have also implicated HCMV infection (based only on increased antibody titer) as a trigger for the development of Guillain-Barré syndrome in solid organ transplant recipients[38].

The "indirect effects" descriptor originated because HCMV could not be easily detected in target organs, but recently, using more sensitive methods including *in situ* hybridization and immunohistochemical methods, there is a growing body of evidence indicating that the virus can be detected in these tissues[39]. Thus, a prescient question is whether it remains valid to refer to these effects as indirect, or whether they are further direct effects that can give rise to both acute and long-term system malfunction. Despite this caveat, in this review we shall continue to use the term "indirect effects" and will focus on the evidence for the involvement of HCMV in these conditions and also how the extended suppression of viral replication through prophylaxis can provide further evidence for HCMV's explicit involvement.

Human cytomegalovirus and graft function in the short and long term

Potentially, and in reality, there is a complex relationship between HCMV replication, organ rejection, and augmented immunosuppression for the management of an acute rejection episode. Thus, if acute organ rejection occurs in the absence of viral replication, the treatment may itself lead to an increased risk of viral replication and an apparent association between HCMV and rejection. Alternatively, if the rejection episode is actually due to micro-infection with HCMV at the organ level, which is treated with enhanced immunosuppression, then this may lead to an exacerbation of the viral infection. It follows that temporal studies are required to disentangle such scenarios, preferably studies that probe replication in the affected organ and in the blood rather than just the latter. Needless to say, studies combining these approaches have been very rare. Fortunately, there is a substantial body of work with the rat CMV organ transplant model, which contributes to our understanding of the probable role that human CMV plays in acute and long-term graft malfunction. The work of the groups of Lautenschlager and Bruggeman, amongst others, have shown that rat CMV infection of animals receiving kidney, liver, heart, aortic, and lung grafts results in enhanced acute and chronic rejection, including bile duct destruction after liver transplantation[40-44]. In these and other studies, rat CMV infection invariable reduces time to graft loss and also accelerates the occurrence of poor graft function as measured by creatinine level post-kidney transplantation[45]. Histopathologi-

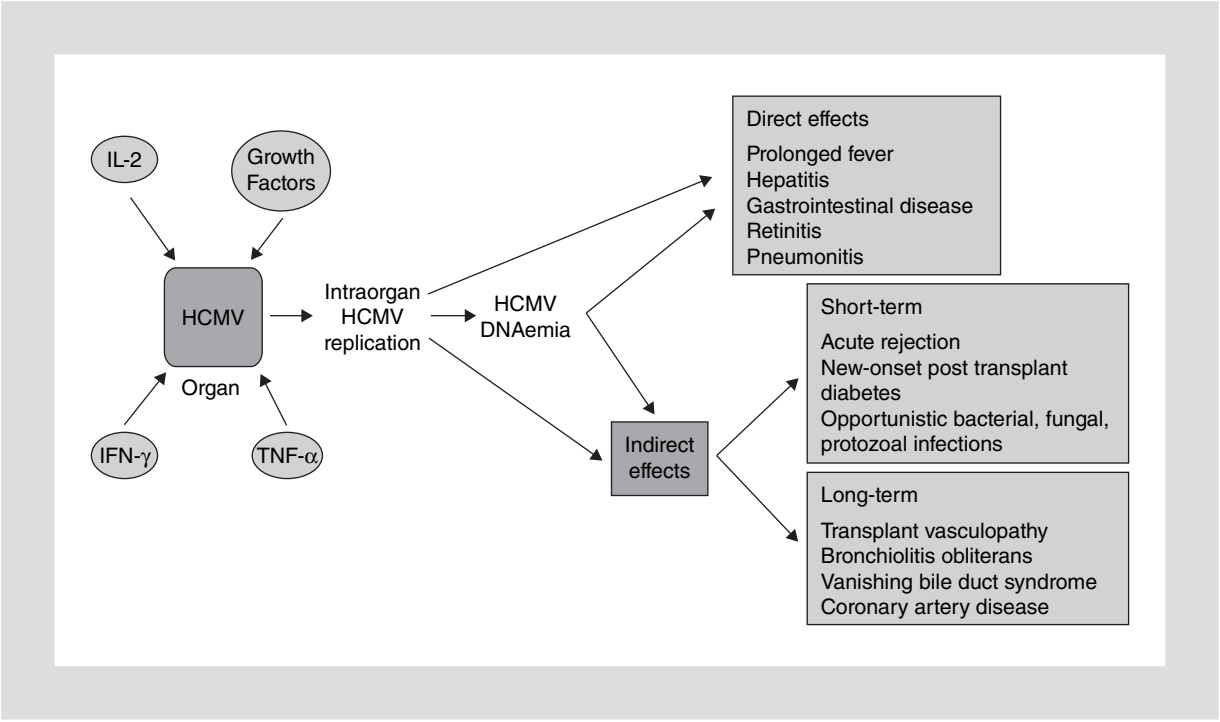

Figure 1. *Schematic diagram showing how latent HCMV in the transplanted organ can be reactivated through a number of proinflammatory cytokines and growth factors. Infection can remain localized in the organ, contributing to a range of direct and indirect effects, or lead to HCMV DNAemia which will allow other organ involvement. The reader should note the differentiation of the indirect effects into those occurring in the short term (typically within three months posttransplant) and those occurring over a much longer timescale.*

cally, extensive vascular injury and fibrosis is noted in the rat CMV-infected animals within 28 days of transplantation, usually leading to terminal rejection[45]. In some studies where rat CMV infection in the transplanted organ has been investigated, it has been shown that rat CMV is present in the allograft from early on posttransplantation, but that it does not necessarily persist at high levels[43]. Rat CMV can induce tubular apoptosis via TNF-TNF receptor 1 in rat models of chronic rejection[46]. Importantly, treatment of rat CMV-infected animals with ganciclovir negates these effects. For example, in the heart transplant model, ganciclovir therapy reduces intimal thickness scores to the levels seen in allogeneic transplants in the absence of rat CMV infection[47].

In the human setting, data linking HCMV with acute and chronic rejection has been more controversial, partly for the reasons outlined at the beginning of this section. Thus, many studies have noted associations between HCMV infection and disease and rejection, but few have addressed the temporal nature of virus replication and rejection. A prospective study by Sagedal, et al.[47] of 477 consecutive renal allograft recipients, who were not receiving anti-HCMV prophylaxis, showed that acute rejection was associated with both HCMV infection (ODDS ratio [OR]: 1.6; p = 0.02) and HCMV disease (ODDS ratio [OR]: 2.5; p = 0.01). Work in our centre has showed in prospective monitoring studies in blood and in the organ that HCMV DNAemia was associated with an increased incidence of acute rejection, with ~ 50% of biopsies showing evidence of HCMV DNA by *in situ* hybridization[48]. The greatest proportions of biopsies were positive for HCMV DNA in the first 20 days posttransplant and intra-organ HCMV preceded DNAemia by around two weeks. Nevertheless, HCMV DNA could be found in biopsies where histologic acute rejection was both present and absent, indicating that a number of factors likely contribute to the clinical picture of rejection. The question arises as to whether the detection of HCMV DNA in these biopsies has any long-term consequences for graft function. This has been addressed in an elegant study by Helantera, et al.[49] who showed that renal transplant recipients with persistent intra-graft HCMV DNA had reduced graft survival and creatinine

clearance at both one and two years posttransplant with odds ratios of 5.1 and 4.3, respectively. Of note was that in multivariate analysis, HCMV DNA in the graft was the only risk factor for lower creatinine clearance at two years, with an odds ratio of 4.9. Other workers have also shown that HCMV infection and disease are associated with chronic allograft nephropathy. For example, Boratynska, et al.[50] analyzed the influence of HCMV on acute rejection and long-term graft function in two renal transplant populations stratified according to immunosuppressive therapy (group 1 received cyclosporine A, azathioprine, and prednisolone, and group 2 received a calcineurin inhibitor, mycophenolate mofetil, and prednisolone). Serum creatinine levels were significantly elevated at six months in patients within both subgroups who had HCMV infection versus those who remained HCMV replication negative. The combination of an acute rejection episode and HCMV disease resulted in elevated creatinine levels at both six and 12 months posttransplantation. The importance of considering the confounding effects of immunosuppressive therapy on HCMV replication should not be underestimated, and we have recently shown some profound differences in the relative rate of HCMV infection in liver transplant recipients receiving different immunosuppressive regimens[51]. A similar role for HCMV as a factor for acute rejection has been observed in the Euro-SPK study of simultaneous pancreas-kidney transplant recipients[52]. In patients with HCMV infection, 66% suffered acute rejection compared to only 44% in the HCMV-free group. The effects of prophylactic ganciclovir in this study will be discussed later in this review.

In the liver transplant setting, associations between HCMV infection and graft rejection have been demonstrated. In studies where detailed intra-organ analyses have been performed, HCMV DNA can be detected at an early stage posttransplant, while classical histopathologic HCMV inclusions are a rare observation consistent with the high intra-organ load needed for this classical appearance of HCMV[53]. One study has shown that this micro-infection in the organ was associated with a significant increase in the expression of a number of adhesion molecules, including intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 and their ligands lymphocyte function antigen (LFA)-1 and very late antigen (VLA)-4, although there was no association with long-term clinical outcome[54].

Human cytomegalovirus and transplant vascular sclerosis

Transplant vascular sclerosis, also called transplant-associated arteriosclerosis and transplant vasculopathy, has been identified as the most important cause of graft failure following the first posttransplant year. Although transplant vascular sclerosis is the hallmark of chronic rejection in cardiac transplantation, it also manifest following transplantation of all solid organs including liver, kidney, and small bowel. Transplant vascular sclerosis can affect the whole length of the vessel and it is characterized by concentric neointimal smooth muscle cell proliferation, resulting in vessel occlusion and ultimately graft failure[55].

A number of clinical studies suggested that HCMV significantly accelerates transplant vascular sclerosis and chronic rejection in solid organ allografts. Grattan, et al. showed that graft atherosclerosis occurred more frequently and was significantly more severe, as judged by angiographic criteria or by pathology, in the HCMV-infected heart transplant recipients than in noninfected recipients[56]. Similar findings were subsequently reported by other investigators[56-63]. In renal transplant recipients, some studies[64,65] found a strong correlation between HCMV disease and atherosclerotic vascular events, whereas Hernandez, et al. found no link between HCMV disease and ischemic heart disease in a similar cohort of patients[66].

The mechanism by which HCMV infection accelerates transplant vascular sclerosis may involve a direct effect of HCMV, the recipient's immune response to HCMV, or an interaction of HCMV and the recipient's alloreactivity to donor tissue. The HCMV infects cells that are important in the pathogenesis of vascular diseases: endothelial cells, smooth muscle cells, and monocytes/macrophages. The HCMV infection of endothelial cells can release viral proteins, which can then be processed by other endothelial cells and presented to the HCMV-specific $CD4^+$ T-cells circulating in the peripheral blood. The production of IFNγ and TNFα causes upregulation of several adhesion molecules on the surface of endothelial cells, such as ICAM-1 and VCAM-1[67-69] and fractalkine (CX3CR1)[70], which contribute to leukocyte adhesion. These chemokines can be also secreted to form a gradient to induce migration of monocytes and natural killer

(NK) cells (as reviewed[71]). Recently it has been shown that antibodies against CX3CR1 reduce chemo-attraction and protect against damage[72]. The HCMV also induces the production of cytokines, growth factors, and extracellular matrix components involved in the process of angiogenesis and wound healing, which are likely to contribute to and accelerate the vascular disease[73]. The migration of smooth muscle cells from the media to the intima space and their subsequent accumulation constitutes a hallmark of the vascular lesion. The HCMV could contribute to this process by encoding inhibitors of cell death and by sequestering the tumor suppressor gene p53 and therefore contributing to the accumulation of smooth muscle cells. Furthermore, the production of fibroblast growth factor and platelet-derived growth factor by infected endothelial cells can also contribute to the proliferation of smooth muscle cells[74]. Following the infection of smooth muscle cells by HCMV, the expression of virally encoded chemokine receptor US28 stimulates cellular migration toward the site of the vascular injury[75,76]. A direct link between HCMV infection and local inflammation in the vasculature, which is a key element in the development of atherosclerosis, has been recently shown by Qiu, et al.[77]. These authors have demonstrated that HCMV infection of human vascular smooth muscle cells is able to directly contribute to the inflammation process by inducing the expression of 5-lipoxygenase and the subsequent production of leukotriene, which in turn promotes leukocyte infiltration.

The HCMV infection of macrophages contributes to the formation of foam cells, which are a key element in the initial formation of the atheromatous plaque. Carlquist, et al.[78] have demonstrated that HCMV infection of tryptophan hydroxylase-1 monocytes/macrophages cell line induces the expression of scavenger receptor CD36, which binds to oxidized low-density lipoprotein and contributes to foam cell development.

Human cytomegalovirus, the immune system, and other pathogens

Immune modulation mediated by human cytomegalovirus

Human cytomegalovirus devotes a large part of its genome to produce proteins which have the ability to interfere with all arms of the immune response. The HCMV encodes four glycoproteins (US2, US3, US6, US11), which can interfere and disrupt major histocompatibility complex (MHC) class I antigen production, with the net result of decreasing the cell-surface expression of MHC class I (reviewed[79]). Two of these glycoproteins also modulate the expression of MHC class II; gpUS2 is able to impede the translocation of MHC class II gene products[80] and gpUS3 prevents antigen presentation by disrupting the invariant chain interaction within intracellular compartments (reviewed[81]).

Human cytomegalovirus has evolved several strategies to modulate NK cell responses. A total of six genes (UL16, UL18, UL40, UL83, UL141 and UK142) and a micro RNA in the UL12 have been characterized to date (reviewed[82]). The HCMV encodes a viral interleukin-10 (cmvIL-10) homolog of the human IL-10[83], which can inhibit immune cell proliferation, inflammatory cytokine synthesis, and MHC class I and II antigen expression[84]. Also, HCMV contains four open reading frames with homology to G-protein-coupled receptors, one of which (US28) shares homology to the CC chemokine receptor CCR1 and is capable of binding macrophage inflammatory protein-1α, monocyte chemoattractant protein-1, and regulated on activation, normal T-cell expressed and secreted (RANTES) and the CX3C chemokine fractalkine 8[85,86], and inhibits chemokine-mediated monocyte migration by sequestering CC chemokines monocyte chemoattractant protein-1 and RANTES produced by infected cells[87]. Another protein, pUL21.5 that is secreted also selectively binds RANTES[88].

Human cytomegalovirus can also interfere with dendritic cell function, therefore preventing the delivery of the signals required for T-cell activation. Thus, HCMV-mediated impairment of dendritic cell function may contribute to virus-mediated immunosuppression[89-91].

Despite this large array of immune evasion strategies, a strong $CD4^+$ and $CD8^+$ T-cell immune response is elicited upon primary HCMV infection, and it is able to control the viral replication while HCMV persists in the host in latent form[92]. Interestingly, and in contrast with the general view of HCMV as the facilitator of other opportunistic infections, herpesvirus latency in the mouse model has been recently found to be associated with host resistance to some bacte-

rial pathogens[93]. Its ability to modulate the expression of the MHC molecules, cytokines, and NK cells has led to HCMV being defined as "immunosuppressive". Indeed, HCMV infection in solid organ transplantation has been shown to facilitate susceptibility to opportunistic bacterial and fungal infections[94-98] although the mechanistic basis for these effects has not been fully elucidated. Consistent with these observations is the demonstration that prophylactic treatment for HCMV following solid organ transplantation decreases the incidence of bacterial and fungal infection in meta-analysis[31,99,100].

Human cytomegalovirus and hepatitis C virus

Human cytomegalovirus infection has also been associated with an increased risk of fibrosis progression caused by HCV infection in liver transplant recipients[101-104]. However, this has not been a consistent finding. For example, our group[105] and others[106-108] showed that, at least at one year posttransplantation, HCMV infection does not influence HCV replication and fibrosis outcome. The adverse outcome in long-term allograft survival described in other studies might be explained by variables such as differences in the immunosuppression regimens used for patient management. Although the mechanistic basis for the apparent link between HCMV and HCV pathogenesis is not known, it is plausible that their respective mechanisms to manipulate the immune response might lead to altered pathogenicity, especially within the liver transplant recipient.

Human cytomegalovirus and posttransplant lymphoproliferative disease

Posttransplant lymphoproliferative disease is a severe complication following solid organ transplantation that occurs in 1-20% of transplant recipients. This disease encompasses a heterogeneous group of lymphoproliferative disorders, predominantly B-cells, of which 90% is Epstein-Barr virus (EBV)-driven B-cell lymphoma[109]. Epstein-Barr virus infection, in particular primary EBV infection after transplantation, is recognized as the principal risk factor for the development of PTLD. The use of immunosuppressive treatment is thought to down-modulate the EBV-specific $CD8^+$ T-cells, which are then unable to contain the proliferation of the EBV-infected B-cells. Other chronic infections such as HCMV have been reported as risk factors for the onset of PTLD in liver transplant recipients who developed primary EBV infection[110,111]. Because high levels of IL-10 have been measured in the serum of transplant recipients with PTLD[112,113], it has been suggested that HCMV could contribute to the increased incidence of PTLD by the production of the viral IL-10 by latently infected cells[114].

Human cytomegalovirus and diabetes

New-onset posttransplant diabetes is a common complication following kidney transplantation, with an incidence ranging from 3-44% depending on the studies. Intriguing data suggest that there may be an association between HCMV infection and posttransplant diabetes mellitus[115-118]. The prospective study of a cohort of 160 nondiabetic renal transplant recipients showed a significantly increased risk of new-onset posttransplant diabetes mellitus in patients who developed asymptomatic HCMV infection (26%) compared to patients without HCMV infection (6%)[116]. More recently, two cases of late-onset HCMV infection (i.e. after discontinuation of valganciclovir prophylaxis) associated with the onset of diabetes mellitus after kidney transplantation were reported[118]. The distinct causative relationship is yet to be determined. The possible link between HCMV infection and the development of diabetes mellitus was initially suggested in a case report of a child with congenital HCMV who developed diabetes at the age of 13 months[119]. Other studies[120-123], but not all[122,123], support this association. The HCMV may damage the pancreatic B-cell in various ways, either (i) directly by infecting pancreatic B-cells with cytopathic effects, (ii) by induction of proinflammatory cytokines caused by infection of B-cells or infiltrating leukocytes leading to altered B-cell function or apoptosis, or (iii) by molecular mimicry between viral proteins and autoantigens. Pak, et al.[124] showed that the HCMV antibody can recognize the islet cell-specific protein *in vitro*. In addition, T-cell cross-reactivity was demonstrated between UL57 and GAD65[125]. Interestingly, the rat CMV model also supports the role of CMV in the pathogenesis of diabetes mellitus (as reviewed[126]).

Impact of antiviral therapy on the indirect effects of human cytomegalovirus

An alternative approach to ascertain whether HCMV is intimately involved in the range of indirect effects is to provide definitive data from antiviral intervention studies. However, the study design is important if one does not fully appreciate the pathogenesis. For example, if a prolonged period of viral replication is necessary to give rise to the indirect effects, a preemptive approach is probably unlikely to have a significant impact, whereas if early high-level replication triggers a set of subsequent effects driven by cytokine and other autocrine networks, then both preemptive therapy and prophylaxis may impact on the indirect effects. Suffice to say, very few studies have been specifically initiated to address this issue. Rather, investigators have performed studies to either determine the effects of prophylaxis on the direct effects of HCMV and then analyzed indirect effects as secondary endpoints, or compared preemptive therapy and prophylaxis and then investigated long-term graft function. Nevertheless, these studies have provided important and in some cases unexpected data supporting the role of HCMV replication in the indirect effects. In this context it is worth noting the impact that ganciclovir therapy had on rejection in the rat CMV model systems summarized under the previous heading of "Clinical effects of HCMV after organ transplantation".

One of the earliest studies to show that prophylactic control of HCMV replication impacted on the indirect effects was the study of high-dose valacyclovir (8 g/day) in renal transplant recipients[127]. In the D^+R^- group, valacyclovir therapy reduced the incidence of acute rejection by 50%, with the Kaplan Meier curves diverging relatively early posttransplantation, which is consistent with the *in situ* hybridization data indicating that HCMV replication is occurring at the earliest times posttransplant in the kidney[49] (Emery, et al. unpublished data). Two aspects of valacyclovir study are noteworthy. Firstly, in the D^+/R^- patients, HCMV replication contributed to acute rejection in 50% of cases, i.e. HCMV is a significant contributor to acute rejection but not the only cause, and secondly, the inhibition of HCMV replication in the D^+/R^+ group made no impact on the incidence of acute rejection. Similar reductions (50%) in acute rejection have been observed in nonrandomized studies of ganciclovir prophylaxis in kidney-pancreas transplant recipients[52]. In liver transplant recipients, ganciclovir prophylaxis for three months has been shown to reduce the incidence of acute rejection by approximately 50%[128]. The administration of HCMV prophylaxis with ganciclovir in the heart transplant setting has been shown to reduce the incidence of transplant vasculopathy in a retrospective study[129]. While this study was based on the long-term follow-up of a cohort of patients transplanted in the early 1990s[130] and managed on a prophylactic strategy that would be regarded as suboptimal by today's standards, it provided the first evidence that inhibition of HCMV replication in the early phase after heart transplantation could have a significant long-term benefit on transplant vasculopathy. More recently, this work has been extended by comparing prolonged prophylaxis with an initial period of intravenous (IV) ganciclovir followed by two months of valganciclovir prophylaxis to standard four-week IV ganciclovir therapy in patients undergoing heart transplantation. In the patients receiving prolonged therapy, acute rejection was decreased (relative risk: 0.55; $p = 0.03$) and it was also associated with reduced coronary artery lumen loss (–10 vs. –21%; $p = 0.05$) and vessel shrinkage (–3 vs. –11%; $p = 0.03$)[131]. While this study has some drawbacks since all high-risk transplants (D^+/R^-) received prolonged therapy and standard treatment was reserved for the lower-risk D^+/R^+ group, it provides further evidence for HCMV replication during the early period posttransplantation, giving rise to longer-term pathological consequences.

In the lung transplant setting, Chmiel, et al. have reported that HCMV prophylaxis with ganciclovir or valganciclovir significantly reduced the occurrence of bronchiolitis obliterans[132]. This reduction (from 60% incidence to 43%; $p = 0.002$) and a concomitant increase in patient five-year survival (an improvement of ~ 55%) was impressive for this patient population. Interestingly, anti-HCMV prophylaxis has consistently been associated with improvement in graft survival and patient survival in both meta-analyses and in a range of other controlled studies[36,66,99,100,133].

Concluding comments

Human cytomegalovirus continues to create challenges for the management of the solid organ transplant recipient. The availability of

more sensitive detection methods is providing new insight into the relationship between low-level replication and pathology in the short, medium, and long term. In addition, substantial progress is being made in understanding the molecular mechanisms that underlie the indirect effects of HCMV. With these data in hand, optimal patient management strategies can continue to be refined so that both the direct and indirect effects of HCMV can be minimized, but not at the cost of increasing antiviral drug resistance.

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