

# Cytomegalovirus and Development of Cardiac Allograft Vasculopathy: Evidences and Therapeutic Implications

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## Abstract

**Cardiac allograft vasculopathy remains the major cause of long-term failure of heart transplantation. Cytomegalovirus infection was identified as a major risk factor for cardiac allograft vasculopathy development in pioneering studies, even though the possibility that the virus is only an innocent bystander was not completely excluded. Only recently, convincing clinical and experimental evidences support the hypothesis of a direct involvement of cytomegalovirus in cardiac allograft vasculopathy pathogenesis. In this article, we review the mechanisms and clinical evidences supporting the hypothesis that subclinical cytomegalovirus infection leads to adverse long-term graft outcome by favoring cardiac allograft vasculopathy development. In addition, we discuss data pointing to the need for antiviral approaches designed to suppress subclinical cytomegalovirus activation as a long-term strategy to prevent cardiac allograft vasculopathy and chronic allograft damage.** (Trends in Transplant. 2010;4:108-16)

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## Key words

**Heart transplant. Cytomegalovirus. Cardiac allograft vasculopathy.**

## Introduction

Short-term survival after heart transplantation has greatly improved over the last three decades as a consequence of advances in immunosuppressive therapy and perioperative management. However, improvement

in long-term outcome is still significantly impeded by the consequences of chronic allograft vasculopathy (CAV), the major cause of late failure of the transplanted heart<sup>1</sup>. Although numerous immune-mediated and metabolic risk factors have been identified for CAV progression<sup>2</sup>, to date no effective treatment is available to fully eliminate its related adverse outcomes. Therefore, the main therapeutic strategy against CAV is the prevention and treatment of the factors known to trigger or accelerate the disease<sup>3</sup>. Among these known risk factors is cytomegalovirus (CMV) infection, which plays a key role in CAV progression, possibly through its complex interaction with the host immune system<sup>4,5</sup>. Importantly, strategies that target CMV offer the

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possibility of effective prevention of CAV while also advancing our understanding of its pathogenesis. However, the efficacy of distinct anti-CMV strategies in limiting CAV requires further evaluation, in concert with studies that identify the specific mechanisms by which CMV mediates graft injury. This knowledge is necessary to advance the field and settle the much-debated controversy of whether CMV is an innocent bystander or directly involved in CAV pathogenesis<sup>6</sup>.

This article reviews recent studies that provide evidence in support of the involvement of CMV in the pathogenesis of CAV. Specifically discussed are the implication of new clinical data and mechanistic pathways potentially implicated in CMV-induced allograft damage. Also discussed are the implications of these data that point to the need for chronic suppression of subclinical viral activation as a long-term strategy to prevent CAV and chronic allograft damage.

### **Relevance of cytomegalovirus infection in heart transplantation**

Cytomegalovirus is a member of the  $\beta$ -Herpesviridae family that includes human herpesvirus-6 (HHV-6) and HHV-7<sup>7</sup>. In the general population, CMV is present in peripheral blood monocytes of 50-90% of individuals, but does not normally cause symptomatic disease. In contrast, CMV is the most clinically relevant posttransplant infectious agent, affecting up to 80% of heart transplant recipients, depending on donor-recipient serostatus, intensity of the immunosuppressive regimen, and the diagnostic system used to assay CMV replication<sup>8,9</sup>.

Following transplantation, immunosuppression causes the reactivation of latent virus, or allows *de novo* transmission of CMV from a seropositive donor to a seronegative recipient (D+/R-)<sup>10</sup>. Bidirectional interaction between the suppressed host immune system

and the immune modulating virus itself increases the risk of infection and CMV disease<sup>11</sup>.

Cytomegalovirus infection has both direct and indirect effects. Direct effects, attributed to the CMV syndrome, typically present as prolonged high fever, fatigue, malaise, anorexia, arthralgias and myalgias, and the leukopenia and thrombocytopenia symptomatic of myelosuppression<sup>12</sup>. Tissue-invasive disease manifests as nephritis, hepatitis, carditis, pneumonitis, pancreatitis, colitis, or rarely as retinitis, seen more frequently among patients coinfecting with HIV<sup>13</sup>. Indirect effects of CMV include allograft injury and rejection, and increased risk for the development of Epstein-Barr virus-associated posttransplant lymphoproliferative disorder<sup>12</sup>. Viral disruption of immune responses and damage to endothelial cells are believed to be responsible for many of the indirect effects of CMV<sup>14,15</sup>.

Transplant recipients who develop CMV infection are at increased risk of mortality. Of note, increased risk for overall mortality appears associated not only with tissue-invasive CMV disease, but also with asymptomatic infection, as detected by pp65 antigenemia<sup>16</sup>, supporting the concept that CMV is capable to indirectly promote graft dysfunction.

Taken together, the evidence supports the conclusion that acute CMV disease following transplantation is a predictor of acute morbidity, and is likely to predispose to chronic long-term graft dysfunction. Thus, strategies directed to prevent CMV disease and to balance the burden of immunosuppressive therapy are mandatory for an optimal posttransplant care.

### **Clinical evidence associating cytomegalovirus with chronic allograft vasculopathy**

The association of CMV infection with CAV was first reported over two decades ago,

in the pre-ganciclovir era<sup>17-19</sup>. These early observational studies were conducted at a time when the methods for detection of CMV infection were relatively insensitive, relying predominantly on clinical manifestations of viral disease and confirmation by histology or viral culture. From these observations developed the concept that CMV may not only cause an organ-specific or systemic disease due to direct viral damage, but is also capable of inducing immune activation that targets the allograft and thus indirectly results in acute rejection and CAV<sup>20</sup>.

The advent of antiviral drugs, particularly ganciclovir, led to effective therapeutic strategies for preventing CMV disease and to a reduction in virus-related graft failure, both in experimental models and in heart transplant recipients<sup>21-23</sup>. These advances in antiviral therapy were paralleled by the development of highly sensitive diagnostic tools to detect CMV infection, enabling the identification of a large number of patients who developed subclinical viral infection, hitherto unrecognized by prior methods<sup>24,25</sup>. By monitoring asymptomatic CMV activation in peripheral blood, Emery, et al. reported that the risk of overt CMV disease is proportional to the level of viral DNA detected<sup>26</sup>. These observations linking viral load with acute disease raised the question of whether asymptomatic viral replication may also predict the long-term consequences of CMV infection. Several studies, summarized below, provide confirmatory evidence for this link.

In a large retrospective analysis including more than 400 kidney recipients<sup>16</sup>, Sagedal, et al. showed that in absence of either prophylaxis or preemptive strategies, CMV infection at a subclinical level as detected by pp65 antigenemia was associated with increased risk for overall and cardiovascular mortality. The authors additionally showed that subclinical CMV disease during the first

100 days after transplantation increased the risk for subsequent rejection<sup>27</sup>.

As opposed to a universal prophylaxis strategy, the preemptive strategy involves the administration of antiviral drugs only in those patients who reach a certain threshold level of viral activity<sup>28</sup>. Thus, patients managed by a preemptive strategy develop significantly higher levels of subclinical CMV replication compared to those managed by a prophylaxis strategy. These two distinct approaches have allowed for the comparison of outcomes with respect to asymptomatic infection<sup>29</sup>.

We have shown that in heart transplant recipients managed by a preemptive strategy, asymptomatic CMV infection was associated with increased risk of developing CAV, defined as abnormal coronary remodeling one year after transplantation<sup>30</sup>. In this study, antiviral treatment was administered only to patients who developed > 30 pp65 positive cells per 10<sup>5</sup> polymorphonuclear cells, consistent with the preemptive approach. In a subsequent prospective study undertaken at Stanford, despite universal antiviral prophylaxis with ganciclovir, CMV DNA indicating active infection was detected in over 90% of the patients, who however remained asymptomatic. In the majority of patients developing CMV infection (80%), CMV was detected only after discontinuing prophylaxis, raising the question of the importance of the duration and type of CMV prophylaxis. To address this question, we compared the outcomes in patients receiving a "standard regimen" of intravenous ganciclovir for 28-days, compared to D<sup>+</sup>/R<sup>-</sup> patients who received a more aggressive regimen consisting in three months of (val-)ganciclovir and CMV hyperimmune serum (CMVIG)<sup>31</sup>. Despite being at higher risk for CMV activation because of the serological mismatch, recipients treated with the aggressive regimen showed delayed and reduced CMV infection rates and, most importantly, a reduced risk of acute rejection and CAV as

compared to patients treated with the standard regimen.

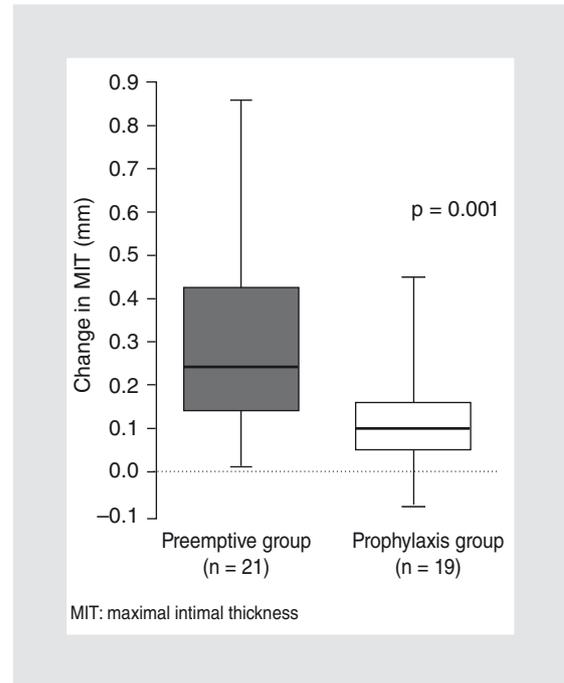
More recently, pursuing the hypothesis that an aggressive anti-CMV strategy could reduce CAV development in CMV-positive recipients, we compared the outcomes of patients managed with a preemptive strategy with a consecutive cohort receiving a 40-day course of valganciclovir, followed by CMV monitoring and additional treatment when patients developed  $> 30$  pp65 positive cells per  $10^5$  polymorphonuclear cells. In this study, the aggressive strategy led to a delayed and reduced magnitude of CMV infection. Most importantly, the prophylaxis-based strategy was associated with a reduced increase in coronary maximal intimal thickness one year after transplantation (Fig. 1)<sup>29</sup>.

Taken together, the findings of these studies suggest a pathophysiological role of CMV in chronic graft failure, limiting long-term outcome in heart transplant recipients. Most important, the recent data suggest that such chronic damage to the graft may progress unabated, even in the absence of overt clinical CMV disease<sup>32</sup>. Randomized clinical trials are required to confirm these observations.

### Possible mechanisms of cytomegalovirus-mediated injury

Cytomegalovirus drives a complex interaction with the recipient immune system that, under conditions of iatrogenic immunosuppression, can promote a local proinflammatory milieu, disrupt tolerogenic mechanisms, and exert immunosuppressive effects. These consequences of CMV infection directly influence the alloimmune response in the transplant recipient and may explain the pathogenesis of CMV-induced CAV.

Interaction of CMV with the host inflammatory response sets the stage for viral



**Figure 1.** Changes in coronary maximal intimal thickness in patients treated with valganciclovir prophylaxis and in those followed by preemptive approach (reproduced with permission from Elsevier)<sup>29</sup>.

replication and active CMV infection. Viral glycoprotein B-mediated virion entry and interaction with host leukocyte toll-like receptors leads to activation of the transcription factor nuclear factor kappa B (NF $\kappa$ B), required for CMV transcription, even in the absence of complete viral particle<sup>33,34</sup>. The NF $\kappa$ B regulates many inflammatory cytokine genes and adhesion molecules and has recognition sites for the CMV major immediate early promoter<sup>35</sup>. Virus-cell interaction is sufficient for activation of NF $\kappa$ B, which is required to initiate the CMV transcription machinery<sup>33,35</sup>. In addition, triggering of NF $\kappa$ B by immuno-inflammatory stimuli (e.g. infections or allograft recognition) contributes to CMV activation in cells harboring latent infection, including monocytes differentiating into macrophages<sup>36</sup>. Of note, allograft transplantation, but not isograft transplantation, induces CMV reactivation in a murine model of latent infection<sup>37</sup>, and pharmacologic inhibition of NF $\kappa$ B may reduce endothelial cell replication of CMV *in vitro*<sup>38</sup>. Activation of NF $\kappa$ B stimulates endothelial cell

replication of CMV. Cytokines and chemokines induced by CMV and released over the course of the immune response activate endothelial cells, resulting in upregulation of adhesion molecules, increased class II major histocompatibility complex protein expression, and the production of cytokines exacerbating allograft inflammation<sup>39-43</sup>. In turn, these events are associated with the development of allograft vascular disease<sup>20,44</sup>.

In addition to stimulating the synthesis of inflammatory mediators by host cells, CMV encodes for chemokine and cytokine homologs. Cytokine homolog genes are believed to favor viral spreading by inducing cell migration and cell proliferation<sup>4</sup>. Particular importance has been ascribed to US28, a viral gene encoding a G-protein-coupled chemokine receptor homolog that induces smooth muscle cell proliferation<sup>45</sup>. In a rat model, deletion of the functional homolog of US28 rCMV led to reduced CMV-dependent transplant vasculopathy<sup>46</sup>. It must be noted, however, that these CMV-dependent inflammatory mechanisms require allogeneic responses to accelerate graft rejection because infected animals receiving syngeneic organs do not develop disease<sup>47</sup>. Therefore, interplay between CMV and the host immune system appears to be a crucial factor in CMV-associated graft injury.

A direct vascular effect of the activation of inflammatory mediators in the graft vascular system is reduced nitric oxide (NO) synthesis by endothelial layer, which rapidly becomes dysfunctional, impairing NO-mediated vasodilation<sup>48</sup>. Endothelial dysfunction is a mechanism preceding and associated with systemic and graft atherosclerosis<sup>49,50</sup>. Of note, CMV infection has been linked to endothelial dysfunction of both graft coronary arteries and systemic vasculature in heart recipients<sup>51,52</sup>. Indeed, chronic endothelial dysfunction, and thus abnormal vascular response to injury, may explain the consistent finding of coronary lumen loss associated with negative remodeling,

instead of intimal hyperplasia, shown by CMV-infected patients<sup>53</sup>. A possible process involved in the induction of endothelial dysfunction by CMV takes into account asymmetric dimethylarginine (ADMA)<sup>53,54</sup>. This ADMA is an endogenous inhibitor of endothelial NO synthesis, increases in conditions of intracellular oxidative stress, amplifies the disruption of endothelial homeostasis and thus may be regarded as a systemic marker of impaired endothelial function<sup>48,55,56</sup>. Cytomegalovirus infection is capable of increasing ADMA in cultured endothelial cells, and patients with CMV DNA detected in peripheral blood were shown to have higher ADMA plasma concentrations and were more likely to develop CAV than recipients with no CMV detection<sup>53,54</sup>.

In addition to being associated with CAV pathogenesis, further negative effects of asymptomatic CMV infection on the peripheral vascular system have been hypothesized by the group from the Great Ormond Street Children's Hospital in London. In pediatric heart transplant recipients, these investigators show that children who experience asymptomatic CMV infection develop chronic endothelial dysfunction in the systemic circulation. These data suggest that the consequences of CMV infection after transplantation may not only be limited to the allograft, and reinforce the concept that subclinical CMV replication may negatively influence later vascular health globally, even when it is no longer detectable in the circulation<sup>51</sup>.

The negative effect of CMV infection on graft tolerance has been elegantly investigated in a recent study by Cook, et al.<sup>37</sup>. In a murine model of heart transplantation where tolerance may be effectively achieved with gallium nitrate treatment, recipients harboring latent CMV not only reactivate the virus, but also develop graft rejection leading to 80% of graft loss, as opposed to the 8% graft loss in CMV-negative recipients. Interestingly, while infiltrating the graft, CMV does not disrupt

graft expression of regulatory genes, nor stimulate allograft-specific immunity, but induces intra-graft inflammatory response mediated by type I interferon upregulation, ultimately leading to graft rejection<sup>37</sup>.

In addition to mechanisms inducing inflammation, CMV exerts several effects relevant to immune-system escape and suppression of cellular immunity<sup>4</sup>, which, paradoxically, may be involved in acute rejection and CAV pathogenesis. In particular, the lack of CMV-specific CD4-positive immunity in CMV-seropositive heart recipients appears to favor earlier onset and magnitude of CMV infection<sup>57-59</sup>. Furthermore, recipients with delayed CMV-specific immunity had also an increased incidence of acute rejection and a more accelerated progression of CAV detected by intravascular ultrasound (IVUS), as compared with those with early CMV-specific immunity<sup>57</sup>. Interestingly, similar findings have been reported also in kidney transplant recipients<sup>60</sup>. Taken together, these data suggest that CMV-specific immunity is protective for preserving graft function, and not induce allograft cross-reactivity. Moreover, we may speculate that the lack of CD4-positive activation is the consequence of a successful CMV strategy of immune system escape that indirectly favors graft injury.

### **Antiviral strategies to prevent transplant atherosclerosis**

Two strategies are commonly recommended for the prevention of CMV infection and disease: universal prophylaxis and preemptive therapy<sup>28</sup>. Their rationales are based on two different preventive assumptions. Prophylaxis almost abolishes viral replication during the first weeks/months after transplantation, when the burden of immunosuppression is higher, thereby delaying the eventual appearance of the infection until a later phase of follow-up, by which time the immunosuppressive burden and risk of rejection is expected

to be lower. As opposed to prophylaxis, a preemptive strategy permits early low-grade viral replication in the belief that it may stimulate the host's own immune response against the virus and will reduce the number of patients needing anti-CMV drugs<sup>61</sup>. A key issue in identifying the optimal strategy for prevention of CMV infection is the choice to limit or to allow asymptomatic CMV replication. In this paragraph, we discuss evidences supporting the graft-related benefit of approaches designed to suppress and prevent asymptomatic CMV replication.

A large meta-analysis of randomized trials evaluating the effect of CMV prophylaxis in solid organ recipients showed that in addition to a clear prevention of CMV disease, the universal prophylaxis strategy was associated to superior survival and lower rejection episodes as compared to placebo<sup>62</sup>. This contrasted with the observations from a meta-analysis of preemptive strategy trials in which the preemptive approach was shown to be effective in preventing CMV disease, but failed to demonstrate any effect on survival or graft-related endpoints<sup>63</sup>. Although it must be noted that in this systematic review, the small sample size of preemptive studies may have hidden its long-term efficacy; in two recent randomized studies, secondary analyses of graft-related endpoints suggested prolonged graft survival in kidney recipients receiving prophylaxis as compared with those followed with a preemptive approach<sup>64,65</sup>. In heart transplant recipients, with a non-randomized design, we have shown that aggressive anti-CMV approaches – based either on a prolonged (val)ganciclovir and CMVIG regimen, or on valganciclovir prophylaxis followed by CMV monitoring and adjunctive treatment – are associated with lower progression of CAV as reflected by vascular remodeling<sup>66</sup> and of intimal hyperplasia<sup>29</sup>.

In addition to antiviral drugs, new lines of evidence regarding the anti-CMV effect of

inhibitors of the mammalian target of rapamycin (mTOR), such as sirolimus and everolimus, offer new strategies to limit the impact of CMV infection on graft function. These two drugs, approved for prevention of acute rejection, have both been shown to reduce the occurrence of CMV infection in solid organ transplant recipients when compared with tacrolimus or mycophenolate<sup>67-70</sup>. Most importantly, both drugs also reduce the progression of CAV by IVUS measurement of coronary artery intimal hyperplasia<sup>71,72</sup>. Of note, the anti-CMV effect of mTOR inhibitors depends on their ability to inhibit the cell proliferation machinery, and not on a direct effect on CMV proteins. Nevertheless, the magnitude of mTOR inhibitors' action in limiting CMV infection appears to exceed the protective effect of valganciclovir prophylaxis. Indeed, in a preliminary observational study<sup>73</sup> including patients receiving valganciclovir prophylaxis or followed by preemptive strategy, we found that those receiving a maintenance immunosuppression regimen that included everolimus developed less CMV infection than those receiving mycophenolate. Importantly, while prophylaxis was effective in reducing CMV infection in mycophenolate-treated patients, patients receiving everolimus had such a low incidence of CMV infection that the advantage of prophylaxis over a preemptive strategy was no longer apparent. Thus, the use of mTOR inhibitors may represent a potent approach to minimize the risk of CMV reactivation and to limit CAV progression.

Immunomodulatory agents provide yet another strategy for preventing CMV infection, reducing its effects on allograft injury. We and others have shown that the combination of ganciclovir with CMVIG appears superior to ganciclovir alone in preventing acute rejection and CAV<sup>31,74,75</sup>. Although we cannot exclude that different durations of prophylaxis may be even more important, there is evidence that hyperimmune sera can provide an additional beneficial immunomodulation of host responses,

thereby reducing the risk of both acute CMV disease and rejection<sup>76,77</sup>. In addition to the effects of improved humoral immunomodulation associated with CMVIG therapy, modulation of CMV-specific cellular immunity appears to play a role in preventing CMV reactivation and CMV-mediated graft injury<sup>57</sup>. These data raise the hypothesis that interventions designed to augment increasing CMV-specific immunity (e.g. by development of a vaccine) may provide yet another strategy for protection from CMV infection and CAV.

Taken together, these studies underscore the concept that aggressive limitation of even subclinical CMV infection with strategies based on prophylaxis with antiviral agents and/or hyperimmune sera and on maintenance immunosuppression regimens may effectively protect long-term graft function.

## Conclusions

Although modern antiviral strategies significantly limit the immediate negative impact of CMV disease in heart transplant recipients, a growing body of evidence suggests that subclinical CMV infection leads to adverse long-term graft outcome. Several experimental studies support the hypothesis that the mechanism of CMV-dependent graft dysfunction is mediated by an active disruption of the interplay between graft and host's immune system. Anti-CMV strategies aggressively targeting subclinical infection may effectively limit these effects by means of prophylaxis with antiviral drugs, immune-system reconstitution approaches, or even by the selection of maintenance immunosuppression. However, well designed randomized controlled studies are needed to confirm observational data regarding the benefits of aggressive anti-CMV approaches and to ascertain whether such expected benefits outweigh cost and toxicity.

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