# Indirect Effects of Cytomegalovirus Infection

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## Abstract

Primary cytomegalovirus (CMV) infection often presents as an asymptomatic self-limiting disease in immunocompetent individuals and is followed by latent persistence in different host tissues. However, solid organ transplant (SOT) recipients and patients undergoing allogeneic haematopoietic stem cell transplantation (alloHSCT) are at risk of life-threatening complications caused by CMV infection. Direct effects (or CMV disease) are marked by viral proliferation in a variety of tissues and organs. Clinical manifestations that are observed after SOT and alloHSCT are gastroenteritis, pneumonitis, hepatitis, uveitis, retinitis, encephalitis and graft rejection. In contrast to the direct effects, indirect effects are a consequence of the maintenance of persistent low-level viral replication and have been associated with an increased risk of rejection and graft dysfunction, graft-versus-host disease, accelerated atherosclerosis, opportunistic infections, malignancies, post-transplant diabetes and Guillain-Barré syndrome. This article aims to summarise these indirect effects of CMV, their possible causes and possible treatment strategies.

## Keywords

Cytomegalovirus, indirect effects, immune modulation

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Human cytomegalovirus (HCMV) is a DNA virus that is part of the herpesviridae family. Infections are common, with the worldwide seroprevalence for CMV ranging from approximately 60 to 100%.[1] In immunocompetent individuals, primary CMV infection is often an asymptomatic and self-limiting disease. If symptomatic, it often occurs as a mononucleosis-like syndrome with fever, lymphadenopathy and splenomegaly. Primary infection is followed by persistence in a latent form in different host tissues. There is increasing evidence that CMV can induce alterations of T-cell function in elderly individuals, e.g. oligoclonal T-cell expansions, narrowing of the T-cell receptor repertoire and accumulation of T-cell subpopulations with altered functional activity.[2–5]

However, CMV infection is still a common and life-threatening complication in solid organ transplant (SOT) recipients as well as in patients undergoing allogeneic haematopoietic stem cell transplantation (alloHSCT). These patients are harmed by both direct and indirect effects of CMV infection (see *Figure 1*). Direct effects are marked by viral proliferation in a variety of tissues and organs. Clinical syndromes that are observed after SOT and HSCT are gastroenteritis, pneumonitis, hepatitis, uveitis, retinitis, encephalitis and graft rejection. In contrast to the direct effects, indirect effects are a consequence of the maintenance of persistent low-level viral replication and have been associated with an increased risk of rejection and graft dysfunction, graft-versus-host disease (GvHD), accelerated atherosclerosis, opportunistic infections, malignancies, post-transplant diabetes and Guillain-Barré syndrome.[6–14]

## Immunomodulatory Effects of Cytomegalovirus Infection

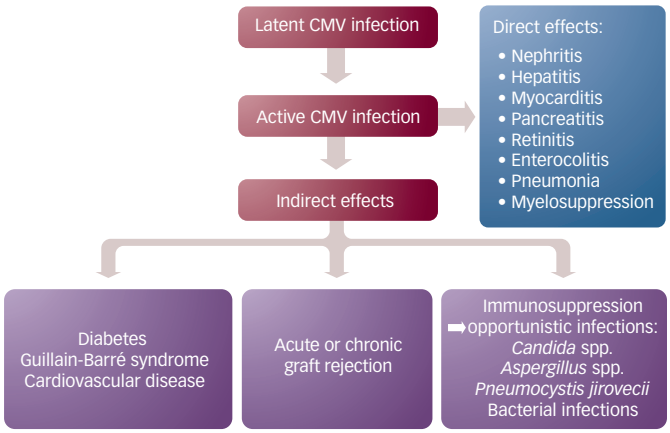
The replication of CMV may cause a variety of indirect effects due to the interaction with the host immune system. In both SOT and alloHSCT patients, the therapeutic effect of immunosuppressive therapy to control rejection may be enhanced by the production of immunosuppressive CMV proteins and the immunosuppressive effects of antiviral agents. Activation of the immune system by viral proteins may lead to the development of rejection and acute or chronic GvHD.

Immunosuppressive effects of CMV infection are caused by CMV replication-induced changes in lymphocytes, monocytes and dendritic cells (DCs) (see *Figure 2*) that change their ability to respond, their ability to produce cytokines and their antigen-presenting capacity.[15,16] T-cell recognition, activation and expansion are altered via different pathways.

This suppression of an adequate immune response is one factor explaining the common association of CMV infection with other opportunistic infections (i.e. invasive aspergillosis, *Pneumocystis jirovecii* pneumonia) or other nosocomial infections, such as fungal and bacterial infections. Further correlation with other herpesviridae has been demonstrated for CMV (e.g. Epstein-Barr virus [EBV]).[17]

Other indirect effects of CMV, such as graft rejection or GvHD, can be explained not by the immunosuppressive effect of CMV

**Figure 1: Direct and Indirect Effects of Cytomegalovirus Infection**




*CMV = cytomegalovirus.*
*Adapted from Fishman et al., 2007.*[41]

replication but by an activation of the immune system as a consequence of CMV replication. Virus replication takes place in a broad variety of cells, resulting in the initiation of inflammatory reactions in the host. This facilitates indirect effects such as graft rejection and/or GvHD. Boeckh and Nichols demonstrate a higher incidence of acute GvHD in CMV-seropositive recipients after alloHSCT compared with seronegative ones. This is in line with activation of the immune system by CMV infection.[18] The relationship between CMV infection and graft rejection seems to be a double-edged sword: CMV is associated with GvHD and rejection, yet rejection and GvHD support CMV replication.

## Indirect Effects of Cytomegalovirus Infection

Some of the indirect effects of CMV occur in transplant recipients in general. Increased risks of acute and chronic rejection and acute and chronic GvHD have been reported. The development of cardiovascular disease has been associated with a precedent CMV infection. Lowance et al. pointed out that CMV infection may influence the risk of acute graft rejection after SOT. Valacyclovir was employed for CMV prophylaxis in patients after renal transplantation. The risk of acute graft rejection was reduced by the prophylactic administration of valacyclovir in CMV-seronegative recipients receiving a graft from CMV-seropositive donors (26 versus 52%; p=0.001).[19]

Epidemiological, pathophysiological and therapeutic results support the fact that CMV infection also contributes to post-transplant atherosclerosis in heart transplant recipients.[20–22] To our knowledge, no prospective studies have yet been performed that explore this clinical phenomenon. The immunomodulatory effects of CMV promote the onset of opportunistic infections and other complications, such as EBV-associated lymphoproliferative disease,[6,7,11] leading to strong immunosuppression.

This effect of CMV infection is supported by the fact that valacyclovir prophylaxis in renal transplant recipients reduces the risk of fungal and bacterial infections such as *Candida* (10% valacyclovir versus 22% placebo; p=0.04) and staphylococcal infections (12 versus 21%; p=0.07).[19] Other groups demonstrated an increased risk of fungal infections in heart transplant patients without ganciclovir prophylaxis[23] and a reduced risk of bacterial and protozoal infections in patients receiving CMV prophylaxis.[7]

Apart from these complications, CMV infection also promotes the development of non-infectious complications such as diabetes and Guillain-Barré syndrome in SOT recipients.[13,14] Even though immunosuppressive treatment is the most frequent cause of diabetes in SOT patients, with an incidence of 3–45%, CMV infection is another important risk factor for the development of post-transplant diabetes.[14] Guillain-Barré syndrome has also been associated with CMV. Experimental data proposed immunological mechanisms for the development of this disease and suggest that infections such as CMV and campylobacter initiate immune responses, causing neural damage. A probable causative relationship between viral replication and Guillain-Barré syndrome has been reported after SOT.[13] There is also evidence that CMV infection is involved in the development of GvHD after alloHSCT.

Our group has demonstrated the induction of syngeneic graft-versus-host reactivity-like symptoms to be correlated with re-activated viral infections after bone marrow transplantation.[24] Söderberg-Naucler et al.[25] have shown the CMV-associated autoantigen CD13 to be immunogenic during CMV infection in bone marrow transplant patients. In their study, all of the 14 patients who had antibodies to CD13 developed either limited or extensive chronic GvHD. The use of pre-emptive CMV therapy was associated with a reduced risk of extensive or severe chronic GvHD in multivariate analysis.[26]

## Prophylactic and Pre-emptive Therapy for Cytomegalovirus Infection

There are two main strategies for the management of CMV infection: prophylaxis or pre-emptive therapy. For prophylaxis, all patients at risk receive antiviral treatment independent of viral replication. In the pre-emptive setting, patients receive antiviral therapy only when there is evidence of CMV replication detected by laboratory assays, such as polymerase chain reaction (PCR)-based assays or pp65 antigen detection.

> *The different courses and manifestations of cytomegalovirus (CMV) disease influence the therapeutic approaches to preventing or treating CMV infections or reactivations and also its direct and indirect effects.*

The course of CMV disease differs among transplant populations such as SOT and HSCT. Recipients of an allogeneic stem cell graft continue to show a high mortality from CMV disease. CMV-seropositive patients, after kidney transplantation, have an increased risk of CMV disease only when receiving intensified immunosuppressive therapies such as pan T-cell antibodies. The different courses and manifestations influence the therapeutic approaches to preventing or treating CMV infections or reactivations and also its direct and indirect effects.[27,28]

## Prophylaxis and Pre-emptive Therapy for Cytomegalovirus and Their Influence on Indirect Cytomegalovirus Effects

Although increasing data are available regarding different therapeutic strategies and their efficacy in CMV disease, there are still many questions left concerning these therapeutic regimens and their impact on indirect CMV effects.

As confirmed by Khoury et al., pre-emptive therapy allows low-level asymptomatic CMV replication to occur.[29] A disputable benefit of pre-emptive antiviral chemotherapy can be anticipated in terms of the indirect effects of CMV infection.[30,31] Prophylactic treatment regimens suppress viral replication and may be more efficient in preventing both CMV disease and life-threatening indirect CMV effects, such as acute graft rejection or higher incidence of opportunistic infections. When treated with ganciclovir or valganciclovir, this has been at the expense of an increase in late-onset HCMV disease[32,33] due to a delayed recovery of the cellular immune response. Some antiviral drugs induce neutropenia and further inhibitory effects on T cells, which may increase the risk of secondary infections.[12,34]

Evidence to date does not allow us to determine whether prophylactic therapy would be superior to pre-emptive therapy with regard to indirect CMV effects. Further studies are needed to clarify this question since pre-emptive therapy is mainly applied in patients at an intermediate risk of CMV disease (CMV-seropositive transplants) bearing an elevated risk of complications such as graft rejection or opportunistic infections. There are few data available supporting the hypothesis that prophylactic therapy decreases the risk of graft rejection. A 50% reduction in the rate of graft rejection was observed in patients who received CMV prophylaxis.[18] Two meta-analyses[7,35] came to the conclusion that CMV prophylaxis has no significant influence on acute rejection or graft loss in SOT patients. One of these studies[7] demonstrated a significant reduction of bacterial and protozoan infections when the patients received CMV prophylaxis. Currently available data may lead to the suggestion that prophylactic therapy may be beneficial for preventing the indirect effects of CMV infection. However, with available antiviral agents there is no evidence that prophylactic therapy is superior to pre-emptive treatment regarding overall survival and morbidity.

*Due to the limited number of agents with anti-cytomegalovirus efficacy, both prophylactic and pre-emptive therapies are applied and no clear advantage can yet be demonstrated for one of the therapies.*

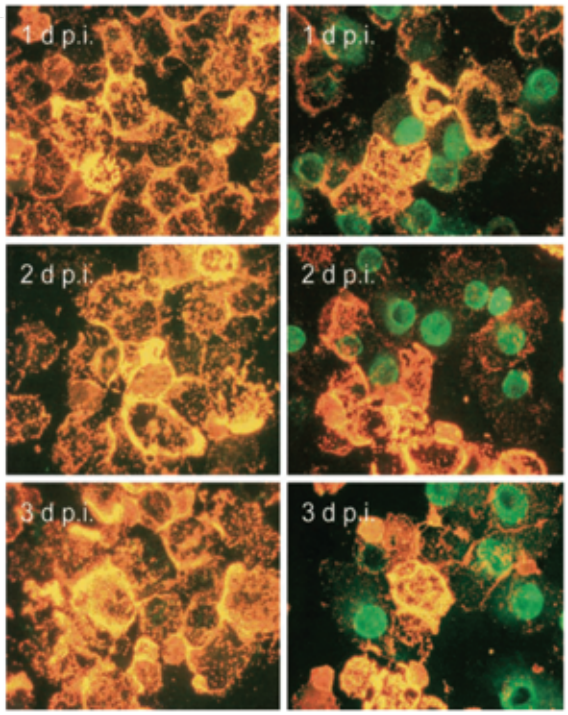
## Summary

The indirect effects of CMV infection in the immunocompromised host remain an important topic for future research projects. These effects are associated with low-level viraemia that might interact with the host immune response and cause serious life-threatening complications such as an increased risk of graft rejection or dysfunction and a high incidence of opportunistic infections. In principle, CMV prophylaxis, by suppressing low-level viraemia, should be able to prevent both the direct and indirect effects of CMV infection and might be the antiviral strategy of choice regarding indirect CMV effects.

Prolonged administration of the current antiviral agents (e.g. ganciclovir) inhibits CMV specific immune recovery and facilitates the occurrence of late-onset CMV disease after discontinuation of CMV prophylaxis. Not only is CMV infection one of the most important and life-threatening complications in the immuno-compromised host, it is also a target well-suited for cellular immunity.[36] Immunotherapeutic approaches provide an attractive

**Figure 2: Inhibitory Effects of Cytomegalovirus Infection on Immature Dendritic Cells[16]**

A




B

| Number | Virus | MHC Class 1 | CD80 | CD86 | CD40 |
|---|---|---|---|---|---|
| 1 | Mock | | | | |
| | TB40/E | ↓ | ↓ | ↑ | ND |
| 2 | Mock | | | | |
| | TB40/E | ↓ | ↓ | ↑ | ND |
| 3 | Mock | | | | |
| | TB40/E | ↓ | ↓ | ↑ | ↓ |
| 4 | Mock | | | | |
| | TB40/E | ↑↓ | ↓ | ↑↓ | ↓ |
| 5 | Mock | | | | |
| | TB40/E | ↓ | ↓ | ↑ | ↓ |
| 6 | Mock | | | | |
| | TB40/E | ↓ | ↓ | ↑ | ↓ |

*↓ = downregulation in infected dendritic cells (DCs); ↑ = upregulation in infected DCs, ↓↑ = no alteration; ND = not determined; mock = uninfected DC; TB40/E = DC infected by human cytomegalovirus (CMV) strain TB40/E.*
*A: Major histocompatibility complex (MHC) class I molecules are downregulated post infection (p.i.) with the CMV strain TB40/E double immunofluorescence* in situ *analysis of MHC molecules (yellow) and viral antigens (green). B: Comparison of immunomodulatory surface molecules in CMV-infected versus mock-infected immature DCs by fluorescence-activated cell sorting (FACS).*

tool both to control CMV infection and to minimise the adverse effects caused by antiviral drugs (ganciclovir: myelosuppression; foscarnet: renal toxicity) or drug resistance. Immunotherapeutic approaches include the transfer of CMV-specific cytotoxic T-lymphocytes[37,38] or a vaccination with antigen-pulsed professional antigen-presenting cells.[39] Novel antiviral agents without myelosuppression or renal toxicity (e.g. maribavir)[40] may, if the ongoing studies confirm safety and efficacy for CMV prophylaxis, offer new possibilities in the prophylactic treatment of CMV infection in the immunocompromised host.

CMV infection leads to a variety of direct and indirect effects. Due to the limited number of agents with anti-CMV efficacy, both prophylactic and pre-emptive therapies are applied and no clear advantage can yet be demonstrated for one of the therapies. Ongoing and new prospective studies will help to clarify the role of prophylactic, pre-emptive and CMV-directed immunotherapy not only to control CMV infection but also to prevent indirect CMV-related complications. ■

Götz Ulrich Grigoleit is a senior physician in the Stem Cell Transplant Unit at the Julius Maximilians University in Würzburg. His scientific work is mainly focused on the specific immune response to cytomegalovirus (CMV) and self-antigens after allogeneic haematopoietic stem cell transplantation (alloHSCT). He has conducted immunotherapeutic clinical trials to control CMV infection after alloHSCT by dendritic cells and specific T-cell transfer. His main experience is in T-cell immunology and modelling of immune responses post-alloHSCT to mediate disease control.

Markus Kapp is a resident physician in the Stem Cell Transplant Unit at Julius Maximilians University in Würzburg. His main focus is on the establishment of methods to monitor tumour-associated-antigen (TAA)-specific T-cell responses. Furthermore, he has evaluated the impact of $CD8^+$ T-cell responses directed to TAAs in disease control after allogeneic haematopoietic stem cell transplantation (alloHSCT). Dr Kapp's main experience is in T-cell immunology, especially the monitoring and modelling of immune responses post-alloHSCT to mediate disease control.

Hermann Einsele is a Full Professor of Internal Medicine and Director of Medical Clinic II at the Julius Maximilians University in Würzburg. He is an active member of various scientific organisations and Chairman of the German Study Group on Multiple Myeloma. In 2003, he received the van Bekkum-Award from the European Society of Blood and Marrow Transplantation. His research interests include multiple myeloma, stem cell transplantation and T-cell therapy.

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