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Immunologic Response and Pathogenic Mechanisms of Cytomegalovirus Infection in Transplant Recipients

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Abstract

Immunity mechanisms of cytomegalovirus infection control have been studied recently and have contributed to the better understanding of the pathogenesis of this disease in patients with a solid organ transplant.

These studies suggest that a relationship exists between cytomegalovirus and the development of acute rejection. This relationship is more evident if the patient develops cytomegalovirus disease and if the donor is cytomegalovirus-positive and the recipient is cytomegalovirus-negative. There is also evidence that the association is probable if there is coinfection with cytomegalovirus and other herpesviruses (human herpesvirus 7).

Over the past 30 years, many attempts have been made to design vaccines able to prevent cytomegalovirus infection and disease; however, few vaccines progressed to clinical studies and none have been licensed yet.

The purpose of this review is to provide a critical analysis of the most recent evidences from the medical literature with respect to the pathogenesis of cytomegalovirus infection and its association with acute and chronic rejection, as well as the possible implications that the development of future vaccines could have in the prevention of cytomegalovirus disease and acute or chronic rejection. **(Trends in Transplant. 2009;3:103-12)**

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Key words

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Introduction

Cytomegalovirus (CMV) is a ubiquitous virus that causes infection, generally in early childhood, and persists for life after primary infection occurs. The majority of these infections are subclinical in healthy subjects; nevertheless, they are an important cause of morbidity and mortality in immunocompromised patients such as solid organ transplant recipients or hematopoietic stem cell transplant recipients[1,2]. The association of CMV infection and the development of acute or chronic rejection has been a matter of discussion in the last decades[3-5].

The CMV genome is composed of lineal, double-stranded DNA, surrounded by a protein lining, called matrix, which contains phosphoproteins (pp65, pp150, etc.) that are highly immunogenic and capable of deregulating the cellular cycle of the host cell. This lining is surrounded by glycoproteins (gB, gN, gO, gH, gM, gL) necessary for the virus infectivity: entrance to the host cell, cell-to-cell dissemination, and maturing[6].

The fusion of the virus with the cell is mediated by the viral glycoprotein gB. The fusion is followed by the entrance of the nucleocapsid and the lining proteins to the host cell cytoplasm; the nuclei are translocated rapidly and pp65 antigen, a marker of infection, is detected in the serum in less than an hour[6]. The main reservoirs of CMV are the fibroblasts, myeloid cells, and endothelial cells[7]. The infection of endothelial cells and macrophages plays an important role in the latency, and this seems to be a critical point in the maintenance of CMV in the host[8].

The start of the replication takes about 12-24 hours after the infection of the cell, and the cytopathic effect in the viral culture could be seen after 7-14 days[9]. As with other herpesviruses, CMV invades the host cell, inhibits protein synthesis, and liberates viral DNA to the nuclei, where the replication starts immediately. A strategy that it shares with other herpesviruses is the ability of stopping the immune response of the host by inhibiting RNA formation, blocking the presentation of antigenic peptides of the cell surface, and blocking apoptosis. These mechanisms prompt to a latent infection that may be reactivated in transplant recipients.

The purpose of this review is to conduct a critical analysis of the most recent evidences from the medical literature with respect to the pathophysiology of CMV infection and its association with acute and chronic rejection, as well as the possible implications that the development of future vaccines could have in the prevention of CMV disease and acute or chronic rejection

Immunity against CMV

Humoral immunity

The protective immunity against CMV is both humoral and cellular. The humoral immunity is important during the viremic phase of infection or reinfection. There are many antibodies directed against the viral glycoproteins (gB and gH) that probably participate in blocking the cell infection[8]. The immunization with these recombinant proteins induces the development of antibodies capable of reducing the plaque-forming units of the virus *in vitro*[10].

The importance of the humoral immunity is suggested by the fact that the severity of the primary infection in seronegative recipients of seropositive donors (D^+/R^-) could be reduced by immunization with the attenuated Town strain vaccine or by the administration of anti-CMV immunoglobulin[6].

The administration of specific immunoglobulin to prevent disease in solid organ

transplant recipients is useful to reduce the incidence of disease[11]. However, humoral immunity by itself cannot prevent the development of disease in the absence of an adequate cellular immunity, as occurs in hematopoietic stem cell transplant recipients[12]. Randomized clinical trials with immunoglobulin in these patients have demonstrated that it is efficacious in reducing CMV infection but not the development of the disease[13-15].

Cellular immunity

The specific T-cell response against CMV is based on their memory function, including their ability in mediating, effectors, proliferation, and secretion of chemokines. The combination of CD4 and CD8 response is critical for the infection control[16].

During primary infection in immunocompetent subjects, the protein pp65 and the protein that codifies the immediate-early 1 exon 4 are the most important targets in the response of the CD8 memory lymphocytes[17]. Other antigens that induce cytotoxic cellular response of the T lymphocytes are the gB protein and the pp150 phosphoproteins, but these are seen only in a third of patients[17]. The cellular response for the immunodominant antigens pp65 and immediate-early 1 exon 4 protein is mediated mainly by the major histocompatibility complex (MHC) class I alleles and therefore induces CD8 lymphocyte response[17,18]. However there could also exist an induction of the CD4 lymphocytes, mediated by MHC class II antigens for the pp65 antigen[17]. As the induction of cytotoxic T lymphocytes (CTL) is mediated by class I antigens, once the induction for the formation of CD8 cells has occurred, the cells only recognize the antigens presented by the same alleles that participated in their induction. So, the subsequent response is not transferable (MHC restriction), and it is feasible to be recovered with autologous cells or lost by cell depletion in early stages of differentiation[19,20]. It has been observed that the incidence of CMV disease in seropositive patients that are selectively given CD34 peripheral blood stem cells is greater than the incidence in patients that receive no selected stem cells (22.6 vs. 4.2%)[21].

Damaging mechanisms and evasion of the immune response

The early replication of CMV in polymorphonuclear leukocytes (PMN) is well known and used as an early diagnostic test: the quantification of the pp65 antigen[23]. The intensity of the expression of this antigen correlates to the quantity of viral particles in blood and the severity of the illness[24,25].

A subsequent step of the replication of the virus in the PMN is the infection of the endothelial cells, which has been demonstrated in patients with prolonged antigenemia for longer than two weeks[54]. In these patients, the intensity of antigenemia has a positive correlation with the intensity of the infection of the endothelial cells. Accordingly, when low levels of antigenemia are found (< 40 cells/200,000 PMN) no endothelial cells infected by CMV are found[26].

At least eight genes are homologous to human proteins related to the immune response[27,28]. This homology helps the virus to evade the immune response. Some of these homologies occur with proteins, as the receptors coupled to the G protein that act as signal transducers mediated by lipids, nucleotides, peptides, and proteins. There are also homologies with the class I heavy chains of the MHC antigens, the beta-2 microglobulin, and even homologous genes of proteins that have chemokine activity[27,28].

The potential role of these genes, homologous to human genes, could be multiple:

(i) in the case of chemokines, favoring the attraction of immune cells that may be infected; (ii) modifying the immune response mechanisms in the presentation of antigens in cases in which there is homology to heavy chains of MHC class I or beta-2 microglobulin; (iii) favoring the lyses of the infected cells and the delivering of viral particles[27].

One of the mechanisms implicated in the permanence of the virus in the infected cell is blocking the stimulating effect of gamma interferon in the presentation of the antigens mediated by the MHC class I antigens[29,30]. Gamma interferon induces the synthesis of the proteins that participate in the presentation of the antigens dependant on class MHC antigens (proteasome proteins, class I heavy chain MHC antigens, β2 microglobulin, TAP1 and TAP2)[32].

Although many of these mechanisms have been studied *in vitro*, the effects could also be observed *in vivo* in an indirect way by following CMV replication kinetics (viral load) and the immune response of the CTL ($CD8^+$) in liver and kidney transplant recipients[34,35]. These studies were performed by two different groups of researchers using the technology named "construction of tetramers", that employs the heavy chain, the β2-microglobulin of class I antigens, a 9 or 10 amino acid oligopeptide, and an enzyme that acts as a signal when linking to the $CD8^+$ lymphocytes specific against CMV[34,35]. In both studies, an increase in CTL is observed when there is an increase of the viral load and afterwards it is controlled. This shows the importance of the cellular immunity to control the infection. In some patients, there was no increase of the CD8 lymphocytes in spite of having a high viral load, and the increase in the specific CD8 lymphocytes was only observed when the viral load decreased with antiviral treatment (ganciclovir). This shows the role of the virus in blocking the immune response.

CMV and rejection

A still unsolved question is the capability of CMV to induce rejection in solid organ transplant recipients.

Immunologic mechanism of rejection

The immune recognition of foreign antigens in the graft is mediated by MHC class I and II. Class I molecules are expressed in all nucleated cells and platelets, while class II are expressed by B lymphocytes, cells of the monocyte-macrophage system and dendritic cells[36]. The T-cells and non lymphoid cells show class II proteins only when they are activated by cytokines[37,38]. Rejection depends on the coordinated activation of T-cells and antigen-presenting cells[37]. For example, in kidney rejection, tubulitis is one of the major diagnostic criteria[39] and consists of the invasion of the tubular epithelium by lymphoid cells. The CD8 lymphocytes are the cells mainly involved in tubulitis development[40]. These cells are attracted by the β-chemokine secretion, especially, MCP-1 and MCP-1β (monocyte chemotactic peptides). Also participating are macrophage inflammatory protein MIP-1α and RANTES (activation regulated peptides expressed in T-cells and possibly secreted)[41].

Something similar occurs in heart transplantation[42]. The expression of self antigens to avoid being recognized and damaged is a constant mechanism[35]. When the expression of class I antigens is less than normal, the natural killer cells cause the lyses of these cells[35]. When the expressed antigens are not recognized by the T-cell receptors as self antigens, the selection of specific clones is

Table 1. Association between CMV and acute rejection

Author	Allograft	Type of rejection	Study design	(n)	Multivariate analysis	Effect of latent CMV infection	Effect of CMV disease
Hodson EM, et al.[61]	SOT	Acute	Meta-analysis	13 RCT		No*	
Kalil AC[63]	SOT	Acute	Meta-analysis	17 RCT		Yes	
Strippoli GFM[64]	SOT	Acute	Meta-analysis	10 RCT		No	
Grattan, et al.[5]	Heart	Acute	Retrospective	301	Yes	Yes	
Teixeira, et al.[52]	Liver	Acute	Prospective	39	No	No	
Toupance, et al.[66]	Kidney	Acute	Retrospective	192	Yes	No	Yes
Boyce, et al.[50]	Kidney	Acute	Retrospective	298	No	No	
Sherlock, et al.[51]	Kidney	Acute	Retrospective	36	No	No	
Lowance, et al.[62]	Kidney	Acute	Prospective	616	No	Yes*	
Kidd IM, et al.[58]	Kidney	Acute	Prospective	52	No	No	Yes†
Reischig T, et al.[65]	Kidney	Acute	Prospective	106	Yes	No	Yes

RCT: randomized controlled trials; SOT: solid organ transplant.
*Lowance, et al. CMV (D+/R–);
†only when there was an association with human herpesvirus 7.

activated to carry out the cytotoxicity, especially by CD8 lymphocytes, affecting the cells that show a greater amount of antigens, such as endothelial or epithelial cells[38,41,42].

Acute rejection seems to be a risk factor for chronic rejection in renal transplantation[43,44]. Nevertheless, many patients experience only one episode of acute rejection and do not have subsequent episodes, but those who have two or more rejection episodes have a greater risk of chronic rejection than those who only have one[45]. This effect is important for graft survival, at least for renal transplants. The survival of the grafts without episodes of rejection is 91% at ten years, while for those with one episode it is 85%, and with more than one episode of rejection the survival of the graft is reduced to 53%[45].

The absence of compatibility of the system human leukocyte antigen 1 (HLA-1) between the recipient and the donor is a very important determinant of rejection. When there are antibodies in the recipient specific against the MHC class I antigens of the donor, the graft is rejected in a fast and irreversible manner[46].

Relationship between CMV and acute rejection

Some clinical studies suggest a possible association between CMV and rejection[47-49]. Nevertheless, it has been difficult to demonstrate with prospective studies that CMV has a direct effect on rejection, although one of these studies demonstrated that in a group of patients, the rejection was reversed when the patients were treated with ganciclovir[40].

Other studies could not demonstrate a relationship between CMV and acute rejection. These authors believe that CMV and rejection are independent events, and their coexistence indicates the general state of the immune response[50-52] (Table 1).

There seems to be an association between human herpesvirus (HHV)-6 and HHV-7 with

CMV and graft rejection. Both viruses have been implicated in the development of graft rejection and graft-versus-host disease[53-55]. The current evidence suggests that there is a temporal relationship between the detection of HHV-6 and HHV-7 and the appearance of CMV infection and also with CMV disease[56]. Kidd, et al.[57] evaluated in a prospective study the natural history of HHV-6, HHV-7 and CMV infection after renal transplantation in 52 patients. The HHV-7 was detected earlier than CMV ($p = 0.05$) and HHV-7 infection was associated to more episodes of rejection ($p = 0.02$). There was more CMV disease in those patients with CMV and HHV-7 coinfection than in those with only CMV infection. The authors concluded that HHV-7 might potentially exacerbate graft rejection. No clear pathologic role was found for HHV-6 in this study. Lautenschlager, et al.[58] retrospectively studied eight liver transplant recipients and documented that an interaction between CMV with HHV-6 and HHV-7 was related to graft rejection. The authors concluded that HHV-6 may infect the liver allograft and cause graft dysfunction and may possibly be associated with rejection and/or CMV infection.

In vitro studies show that the endothelial cells infected by CMV induce the expression of molecules that favor the adhesion of leukocytes to these cells by the induction of the expression of intercellular adhesion molecule 1 (ICAM-1) type molecules, blocking the capability of expression of vascular cell adhesion molecule 1 (VCAM-1) and selecting E, in spite of being stimulated with tumor necrosis factor alpha (TNFα)[59]. The attraction of these cells could favor vascular rejection, but could also favor the dissemination of CMV using the leukocytes as a vehicle.

A meta-analysis that assessed the reduction of the incidence of rejection with acyclovir or ganciclovir prophylaxis showed a significant reduction of the incidence of rejection[60]. A more recent systematic review by Hodson, et al.[61] that evaluated the impact of prophylactic strategies with antivirals in the risk of developing CMV disease and death, did not demonstrate a significant association between CMV infection or disease and acute rejection (RR: 0.90; 95% CI: 0.75-1.05) or graft loss (RR: 0.74; 95% CI: 0.47-1.17), but in one of these essays[62] prophylaxis significantly diminished the risk of acute rejection in CMV-seronegative recipients of kidneys from seropositive donors (RR: 0.51; 95% CI: 0.35-0.74) compared to seropositive recipients (RR: 0.84; 95% CI: 0.63-1.10).

Another meta-analysis[63] assessed the efficacy of universal prophylaxis and preemptive strategies to prevent CMV end-organ disease and other complications in solid organ transplants in 17 trials involving 1980 patients. They found that both universal prophylaxis (OR: 0.74; 95% CI: 0.59-0.94) and preemptive therapy (OR: 0.47; 95% CI: 0.24-0.91) reduced the rate of allograft rejection.

On the other hand, a recent meta-analysis by Strippoli, et al.[64] evaluated the efficacy of preemptive treatment in preventing symptomatic CMV disease (476 patients). Compared with placebo or standard care, preemptive treatment significantly reduced the risk of CMV disease (6 trials, 288 patients; RR: 0.29; 95% CI: 0.11-0.80), but did not reduce the risk of acute rejection (3 trials, 185 patients; RR: 1.06; 95% CI: 0.64-1.76).

Reischig, et al.[65] prospectively evaluated the impact of CMV disease and asymptomatic CMV infection on the development of biopsy proven acute rejection during the first 12 months after renal transplant in patients without CMV prophylaxis. The multivariate analysis showed that CMV disease is associated in an independent way with acute rejection (95% CI: 1.2-7.1; $p = 0.014$). The asymptomatic infection, nevertheless, was not associated to acute rejection. These results are compatible with those of Toupance, et al.[66],

which studied 192 renal transplants and documented an association between acute rejection and CMV disease but not with asymptomatic infection.

In summary, the majority of the studies suggest a relationship between CMV and the development of acute rejection, especially if the recipient is seronegative and receives a graft from a seropositive donor (D^+/R^-). It is possible that the risk increases if there is coinfection of CMV and other herpesviruses (HHV-7).

CMV and chronic rejection

In a study performed with liver transplant recipients[52], CMV infection was associated to chronic rejection in 26% of the patients. Sharing one or two HLA haplotypes and the presence of CMV infection showed an apparent association, but the association of total incompatibility (not sharing any haplotype) and the absence of CMV infection was not analyzed. On the other hand, the effects of CMV infection could simulate chronic rejection[67,68].

There are many interesting data coming from clinical experience that suggest a relationship between CMV infection and chronic rejection. For example, in the group of patients with the greatest risk of developing CMV disease (D^+/R^-), chronic rejection is more frequent when viral infection is prolonged (viral detection longer than 30 days)[68], but this phenomenon was associated to more frequent incompatibility between MHC class I antigens.

Another study with a large number of patients (n = 1339)[69] did not find an association between CMV infection and the subsequent development of chronic rejection. In this study, there was a stronger association, as in other studies, between acute rejection and the development of chronic rejection[70,71].

A mechanism that could explain the association between CMV and chronic rejection is the production during active CMV infection of antibodies against endothelial cells, which could induce endothelial damage and mediate the production of chronic rejection[72]. These antibodies recognize, apart from the antigens of the endothelial cells, other antigens expressed by fibroblasts, keratinocytes, platelets, and mononuclear cells[73].

Use of vaccines

The development of a vaccine to prevent CMV disease is still a challenge. Over the past 30 years, many attempts have been made to design a vaccine able to prevent symptomatic congenital disease and disease in immunocompromised individuals, particularly transplant patients. However, few vaccines progressed to clinical studies and none have been licensed yet. The investigation is directed to find the best antigens with minimum toxicity able to induce both humoral and cellular immunity.

Whole virus live-attenuated vaccines (Towne strain vaccine and recombinant Towne and Toledo strain vaccine)

The Towne vaccine was the first vaccine developed. This is a live-attenuated form of the CMV Towne strain, and has been extensively evaluated since 1975. It induces humoral and cellular immunity to the virus in healthy immunocompetent subjects, but only gives modest protection against CMV diseases and is not able to prevent infection[74,75]. Another live-attenuated vaccine is a chimeric one, based on Towne strain and Toledo strain. A trial with this vaccine on CMV-seropositive adults showed strong T-cell immunity, but

preexisting immunity made it difficult to analyze the results. Phase I trials showed that both Towne and Towne/Toledo vaccines are safe, but extensive long-term clinical trials are necessary to rule out risks regarding the use of live replicating-competent vaccines: prenatal damage, and the problem of latency and reactivation in immunocompromised patients.

Subunit vaccines

Made of the most relevant antigens, either combined with adjuvant or vectored vaccines, these subunit vaccines could overcome the possible problems of long-term safety. They are based on the assumption that immunity directed toward a limited number of antigens is sufficient to induce protective immunity. Purified glycoprotein B plus different adjuvants are being evaluated in animal models and in humans (adults, toddlers, postpartum women, and soon in transplant patients). Another form of subunit vaccine is the vectored one: the gene for the CMV antigen is expressed in a non-replicating vector. The vectors used for these vaccines are canarypox, adenovirus, and vaccine virus Ankara. Canarypox-gB induces both T-cell responses and neutralizing antibodies in animals, but it does not induce sufficient neutralizing antibodies in humans. Recent trials are studying a prime boosting approach combined with other vaccines: two doses of canarypox-gB followed by a dose of Towne vaccine. This trial showed a strong anti-CMV neutralizing antibody response and significant and long-lasting CD4 and CD8 responses.

Other vaccines made with other vectors

DNA vaccines

A bivalent DNA vaccine (pp65 and gB) induces gB-specific neutralizing antibodies and virus-specific CTL responses in a murine model. It also induces antibody and T-cell immunity in CMV-seronegative humans. A trivalent DNA vaccine based on pp65, gB and immediate-early 1 in combination with Towne vaccine has been recently tested.

Other vaccines

(Dense bodies-based vaccines and peptide-based vaccines). Dense bodies are enveloped, replication defective, subviral particles formed during replication in cell culture. They contain the dominant CMV antigens. Dense bodies are a novel, promising approach. Animal studies showed that immunization with dense bodies could induce humoral and cellular responses. Another approach is a peptide-based vaccine with synthetic immunodominant CTL epitopes and/or T-helper epitopes. This vaccine is safe in animal testing and can induce CD8 and CTL response. Finally a poly epitope technology is being investigated (multiple epitopes from eight different CMV antigens)[74-76].

Immune-based treatment

The recovery of CMV-specific T-cell immunity decreases the risk of developing disease. Many attempts have been made to passively restore CMV cellular immunity by infusion of either virus-specific CD8 or a combination of CD4 and CD8. Adoptive immunotherapy is still confined to research centers. Immunomodulatory therapy of CMV pneumonia after liver transplantation has been assayed with good results[77].

In a recent review, Hodson, et al.[78] assessed the benefits and harms of immunoglobulins (IgG), vaccines or interferon for preventing symptomatic CMV disease in solid organ transplant recipients. They conclude that currently there are no indications for IgG or interferon in the prophylaxis of CMV disease

in recipients of solid organ transplants. Although IgG reduced the risk of death from CMV disease in six trials, there was no difference in the risk of CMV disease, infection or all-cause mortality when comparing antiviral medication combined with IgG and antiviral medication alone. There was no significant difference in the risk of CMV disease with anti-CMV vaccine or interferon compared with placebo or no treatment.

Conclusions

The immunity mechanisms and the infection progression studied recently have contributed to the better understanding of the pathogenesis of CMV disease in transplant recipients.

The role of CMV in acute or chronic rejection of the graft continues to be controversial. The evidence in favor of an association between acute rejection and CMV seems to be stronger for CMV disease and the D^+/R^- setting. There is also evidence that supports an association if there is coinfection of CMV and another herpesvirus (HHV-7). Stimulating cellular immunity against CMV could have an important impact in solid organ transplant recipients, reducing the incidence of CMV disease and consequently reducing the associated complications such as rejection.

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