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The Immunology of Posttransplant CMV Infection: Potential Effect of CMV Immunoglobulins on Distinct Components of the Immune Response to CMV

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Abstract: The immune response to cytomegalovirus (CMV) infection is highly complex, including humoral, cellular, innate, and adaptive immune responses. Detection of CMV by the innate immune system triggers production of type I IFNs and inflammatory cytokines which initiate cellular and humoral responses that are critical during the early viremic phase of CMV infection. Sustained control of CMV infection is largely accounted for by cellular immunity, involving various T-cell and B-cell subsets. In solid organ transplant patients, global suppression of innate and adaptive immunities by immunosuppressive agents limits immunological defense, including inhibition of natural killer cell activity with ongoing lowering of Ig levels and CMV-specific antibody titers. This is coupled with a short-term suppression of CMV-specific T cells, the extent and duration of which can predict risk of progression to CMV viremia. CMV immunoglobulin (CMVIG) preparations have the potential to exert immunomodulatory effects as well as providing passive immunization. Specific CMVIG antibodies and virus neutralization might be enhanced by modulation of dendritic cell activity and by a decrease in T-cell activation, effects which are of importance during the initial phase of infection. In summary, the role of CMVIG in reconstituting specific anti-CMV antibodies may be enhanced by some degree of modulation of the innate and adaptive immune responses, which could help to control some of the direct and indirect effects of CMV infection.

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Distinct Components of the Immune Response to Cytomegalovirus

The immune response to primary cytomegalovirus (CMV) infection combines humoral and cellular, innate and adaptive immune responses to limit viral replication and achieve viral latency (Figure 1). The CMV is one of the most complex viruses to infect humans, and the intricacy of both innate and

adaptive immune responses means that it has not yet been fully characterized.

The CMV infection is first detected by the innate immune system via pathogen recognition receptors, well before the onset of adaptive immunity. In vitro studies have demonstrated that Toll-like receptors detect glycoprotein B on the envelope of CMV particles, triggering production of distinct cytokines by immune cells, including type I IFNs and inflammatory cytokines.¹ The CMV induces macrophage TLR4 and TLR5 ligand expression and MyD88 signals related with an inflammatory response with TNF- α , IL-6, and IL-8 gene expression.²

Two studies in liver transplant patients have demonstrated that genetic polymorphisms of the Toll-like receptor 2 gene that disrupt recognition of the CMV glycoprotein B antigen are associated with a significant increase in CMV replication and risk of CMV disease.^{3,4} Separately, recognition of CMV components by the natural killer (NK) cells of the innate immune system stimulates IFN- γ secretion by effector cells. The NK cells express killer cell Ig-like receptors, and greater expression of these activating receptors shows a negative correlation with CMV replication in kidney transplant patients.⁵ There is also evidence for the emergence of memory-like NK cells (CD57⁺NKG2C^{hi} NK cells) within the first two weeks after detection of CMV viremia.⁶ An antibody-mediated response of NKG2Cbright NK cells against human CMV has been recently described, highlighting the important point that the antihuman CMV response may result from cooperation between specific Igs and NK-cell subsets.⁷

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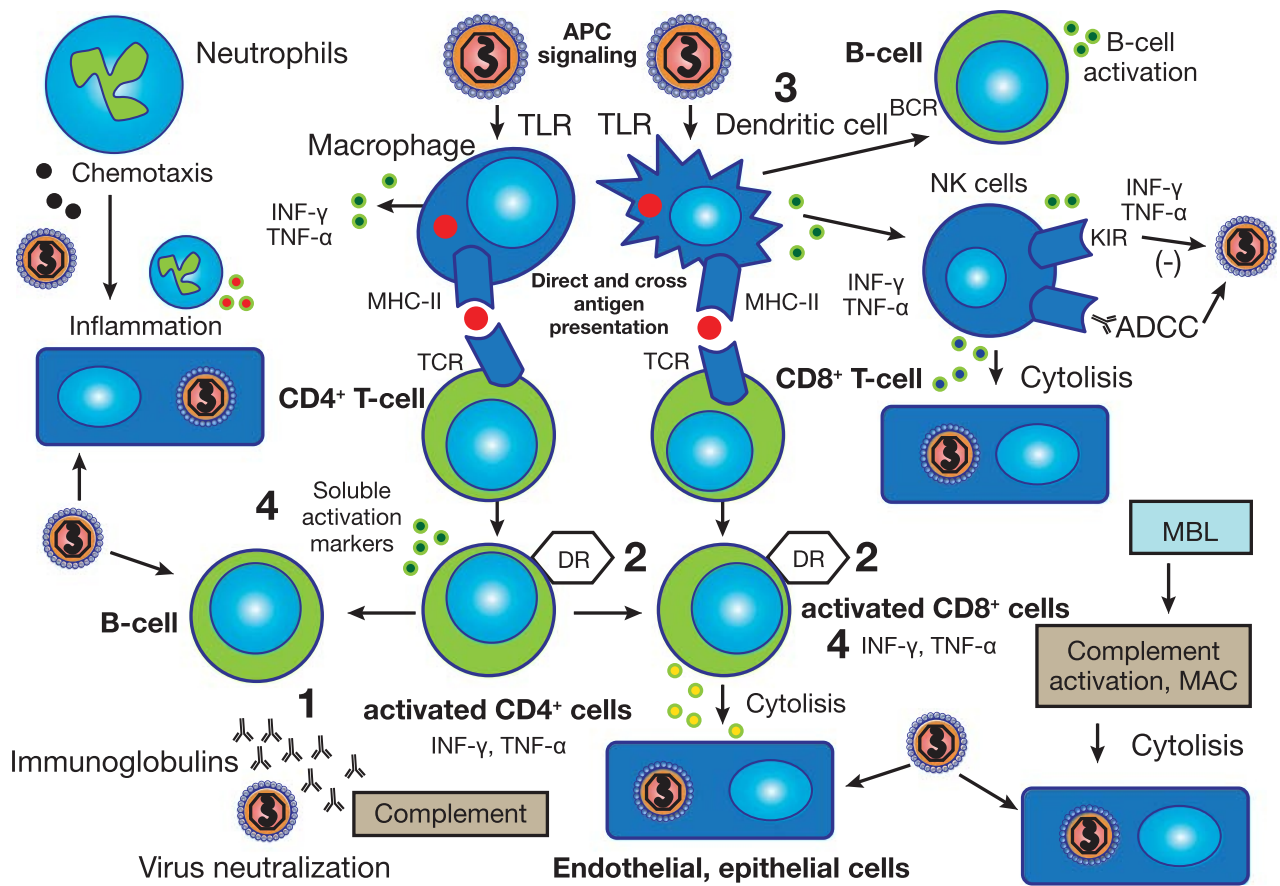


FIGURE 1. The immunological response to CMV. Upper section: Antigen presentation to CD4⁺ and CD8⁺ T cells by macrophages and dendritic cells; participation of other innate immune cells such as neutrophils. Interaction of dendritic cells with B cells and NK cells. Lower section: Cytotoxicity of CMV by CD8⁺ T cells, NK cells, complement system and virus neutralization by immunoglobulins produced by B cells. ADCC, antibody-dependent cellular cytotoxicity; APC, antigen-presenting cell; BCR, B-cell receptor; MAC, membrane attack complex; MHC, major histocompatibility complex; TCR, T-cell receptor; TLR, Toll-like receptors. (1-4) Potential mechanisms of action of CMVIG. (1) Virus neutralization by anti-CMV antibodies, (2) effect on maturation of dendritic cells, (3) decreased T-cell activation, (4) decreased cytokine production.

In murine CMV infection, an unexpected role has been suggested for neutrophils as potent antiviral effector cells which restrict viral replication and the associated pathogenesis in peripheral organs.⁸

Release of cytokines triggered by detection of CMV via the innate system initiates a humoral response during the early viremic phase of CMV infection.^{9,10} In vitro, CMV-specific antibodies emerge in the serum 2 to 4 weeks after the primary infection.¹¹ One of the established targets for neutralizing antibodies is the domain-2-epitope of glycoprotein B on CMV; 1 study in kidney transplantation found that patients with antibodies against this antigen did not require preemptive therapy or develop CMV disease.¹² The CMV-seropositive transplant candidates, by definition, have higher immunocompetency against CMV than seronegative individuals. One comparative analysis of 126 CMV-seropositive versus 19 CMV-seronegative heart transplant patients showed that in addition to a higher pretransplant anti-CMV titer [24 112 versus 453 titer dilutions; $P = 0.001$], the CMV-seropositive patients had higher total IgG levels and CD8 counts.¹³ However, preexisting CMV immunity antibodies may not be entirely effective against CMV strains introduced by organ transplantation. Even in CMV-seropositive kidney transplant patients, receipt of an organ from a seropositive donor increases the risk of infection by up to 3-fold

compared with a seronegative donor.¹⁴ In addition, in vitro studies suggest that neutralizing antibodies may not prevent subsequent rounds of infection that are mediated primarily by direct cell-to-cell transmission after CMV infection has occurred.¹⁵ These findings underline the importance of the cellular immune response in addition to the humoral response.

The role of complement in the clearance of CMV infection is less well studied. Complement proteins are involved in the control of CMV infection in patients with circulating neutralizing anti-CMV antibodies.¹⁶ In the presence of specific anti-CMV antibodies, complement has been shown to enhance the neutralizing ability of serum by 2- to 3-fold.¹⁷

Sustained control of CMV infection is largely accounted for by cellular immunity. At around 4 to 6 weeks after the primary infection, a diverse T-cell response develops in which broadly targeted CMV-specific CD4⁺ and CD8⁺ cells dominate the memory cell subset and are adequate to achieve viral control in healthy individuals.¹⁸ In vivo data have shown that CMV-specific CD8⁺ T cells are largely responsible for the containment of virus-infected cells.¹⁹ Patients with late emergence of IFN- γ -producing CMV-specific CD4⁺ T cells are more likely to develop CMV disease.²⁰ The frequency of CMV-specific cytotoxic CD8⁺ T cells required to effectively suppress viral replication long-term is higher than for other

persistent human pathogens,²¹ reflecting the persistence of CMV infection. The CMV-specific cytotoxic CD8⁺ T-cell counts remain relatively consistent within a subject over time despite considerable interindividual variation.²² Adequate numbers of effector CMV-specific CD4⁺ T cells are also essential for efficient control of replication²³ and, after replication is suppressed, these cells may begin to express cytolytic molecules.²⁰ Combined with CMV-specific antibody titers, this lifelong CMV-specific T-cell immunity controls CMV-related disease in the healthy population. Distinct diagnostic approaches for measurement of CD4⁺- and CD8⁺-specific anti-CMV responses have the potential to help define risk for CMV infection and disease.²⁴ Recently published data have shown that assessment of CMV-specific memory T- and B-cell responses among seronegative recipients before kidney transplantation may help to identify immunized individuals more accurately.²⁵

Evaluation of CMV-specific cellular phenotypes correlates well with serological definition of high risk for development of CMV infection, and with CMV viremia.²⁶ Furthermore, measurement of specific anti-CMV cellular responses are being evaluated to guide immunosuppressive and antiviral therapy.²⁷⁻²⁹

The Immune Response to CMV in Transplant Recipients

Recipients of a solid organ transplant are relatively ill-equipped to mount an effective immune response. Global suppression of innate and adaptive immunities by immunosuppressive agents, particularly lymphocyte numbers and function, is a prerequisite for successful graft survival. Chronic immunosuppression can influence the immune cascade in various ways, with agents often acting synergistically at different points. Calcineurin inhibitors block the maturation of T cells such that B-cell activation by Th1 cells to produce CMV-specific antibodies is suppressed, and production of CMV-specific T cells is reduced, at least in the short term.³⁰ In vitro, calcineurin inhibitors have been shown to suppress CMV-specific T-cell reactivity in a dose-dependent manner.²³ Mycophenolic acid suppresses both T-cell and B-cell functions,³¹ resulting in a higher rate of CMV infection compared with mammalian target of rapamycin inhibitors.^{32,33} Lymphocyte-depleting induction agents, typically suppressing production

of several immune cell types, may be associated with higher risk of CMV infection than nondepleting IL-2 receptor antagonists, especially at high doses.³⁴⁻³⁷ After transplantation, a series of studies has demonstrated profound changes in both the innate and adaptive immune systems related to CMV immunity arising from immunosuppressive therapy.³⁸

Innate Immunity in Organ Transplant Recipients

The NK-cell activity decreases in the month after heart transplantation in patients with or without CMV infection.^{39,40} In a series of 116 patients, Sarmiento et al⁴⁰ demonstrated a pronounced decrease in CD56/CD16⁺ NK cell count by day 7 posttransplant. The decrease was significantly greater in those patients who experienced an infection, with only a partial recovery by month 1⁴⁰ (Figure 2). The same study showed a decrease of approximately one third in levels of C3 complement from baseline to day 7 in patients with CMV infection, and a smaller decrease in infection-free patients; there was near-complete recovery by day 30 only in those patients without infection.⁴⁰ C3 hypocomplementemia has been recently proposed as a risk factor of CMV infection in heart recipients.⁴¹

Polymorphism of mannose-binding lectin (MBL) has been associated with risk for development of CMV infection in solid organ transplantation.^{21,22} A higher frequency of CMV reactivations was observed in lung recipients with deficient versus normal levels of MBL.²³ Lower concentrations of MBL, and the presence of low or intermediate MBL-producing genotypes, are risk factors for the development of CMV infection in heart recipients.^{42,43}

Humoral Response to CMV in Organ Transplant Recipients

An immunological study of 116 heart transplant recipients has shown that Ig levels decrease markedly in heart transplant patients by day 7 posttransplant, with no sign of recovery by month 1.⁴⁰ This ongoing suppression can be directly attributed to the effect of immunosuppression because control patients undergoing nontransplant heart surgery showed full recovery of IgG levels by month 1.⁴⁰ Notably, levels of IgG were significantly higher at baseline and at days 7 and

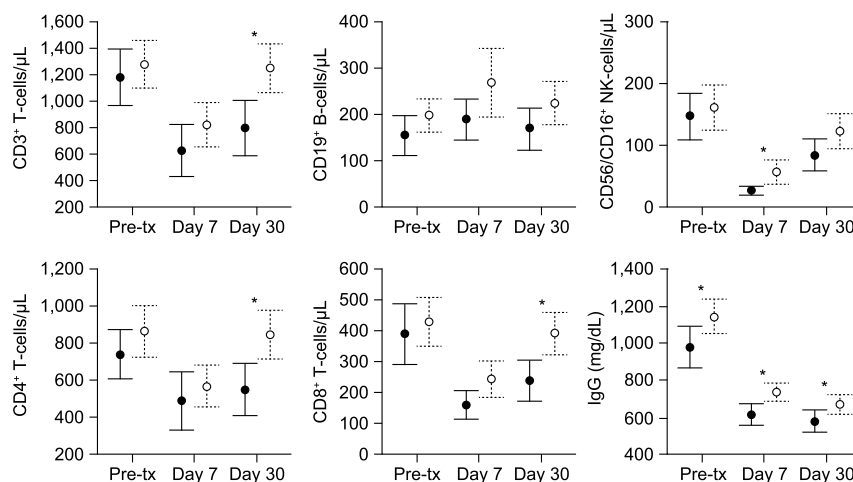


FIGURE 2. Humoral and cellular immunity parameters in 116 heart transplant recipients pretransplant and on days 7 and 30 posttransplant. Dotted error bars (with open circles) represent the error observed in the average of the different immunological parameters in patients without infections, while solid error bars (with closed boxes) refer to patients with infections. Tx, transplantation. * $P < 0.05$ for difference between groups. Reproduced with permission from Sarmiento et al⁴⁰.

30 posttransplant in patients who remained free of severe infection of any type (Figure 2).⁴⁰ Unsurprisingly, given the necessity for lifelong multidrug immunosuppressive regimens, secondary hypogammaglobulinemia is a frequent finding in solid organ transplant recipients.^{44,45} A large pooled analysis undertaken by Florescu et al⁴⁴ recently reported the incidence of hypogammaglobulinemia (defined as serum IgG <700 mg/dL) at 1 year posttransplant to be 49% among heart transplant recipients and 63% among lung transplant recipients, with severe cases (serum IgG <400 mg/dL) in 21% and 22% of patients, respectively. Use of more potent immunosuppressive regimens, particularly the inhibition of T-cell and B-cell functions by mycophenolic acid, may predispose patients to hypogammaglobulinemia.^{31,46,47}

In the pooled analysis by Florescu et al,⁴⁴ the risk of CMV infection during the first year after solid organ transplantation was increased more than 2-fold in patients with severe hypogammaglobulinemia (odds ratio, 2.40; $P = 0.02$). Early hypogammaglobulinemia (≤ 1 month posttransplant) in thoracic transplant recipients is associated with a dramatic increase in the risk of CMV infection (Figure 2). In a prospective, single-center study of 75 heart transplant patients, 10 of whom developed CMV disease during follow-up, multivariate analysis showed a relative hazard of 4.49 for CMV disease ($P = 0.02$) for IgG hypogammaglobulinemia (<500 mg/dL) at month 1 posttransplant.⁴⁸ Using a slightly higher cutoff point of 600 mg/dL at day 30, the same group recently confirmed in a larger population in a multicenter study ($n = 202$) of CMV-seropositive heart transplant recipients that low IgG is an independent risk factor for CMV disease (adjusted odds ratio, 11.9; $P = 0.02$).⁴⁹ Low levels of IgG at day 7 posttransplant are also predictive of infection of any type,^{50,51} consistent with previously published data.⁵¹ Of note, the combination of low levels of IgG coupled with low complement C3 at month 1 confers a particularly high increase in the risk for CMV disease by month 6.⁴⁹

A reduction in CMV-specific antibody titers has also been documented during the first month after heart transplantation, regardless of CMV infection status.^{39,48,49} In a prospective analysis of 202 CMV-seropositive heart transplant patients at a single center, the mean anti-CMV titer decreased from 20 419 to 14 235 titer dilutions by day 30 in patients who developed CMV disease ($P = 0.044$) and from 22 232 to 18 247 titer dilutions ($P < 0.001$) in patients without

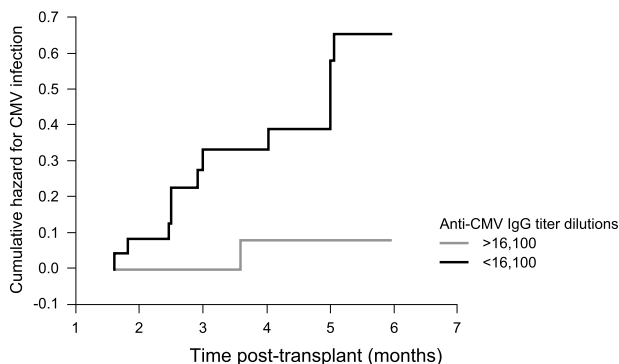


FIGURE 3. Cumulative hazard for CMV infection (Kaplan-Meier estimates) in a prospective study of 38 CMV-seropositive heart transplant patients according to anti-CMV antibody titer. Reproduced with permission from Carbone et al³⁹.

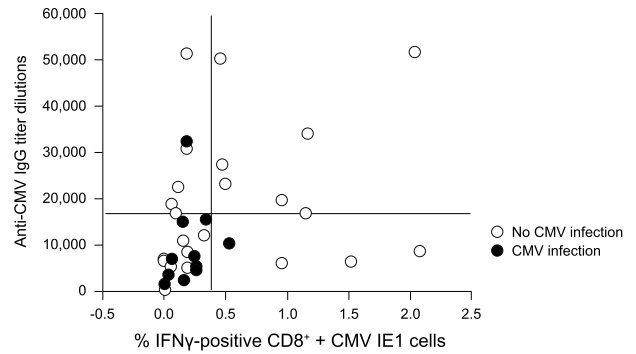


FIGURE 4. Association between the humoral response (anti-CMV antibody titer) and the cellular response (% CMV-specific CD4⁺/CD8⁺ cells) at day 30 posttransplant and presence or absence of CMV infection at month 6 in 38 CMV-seropositive heart transplant patients. IE1, immediate early protein. Reproduced with permission from Carbone et al³⁹.

CMV disease.⁴⁹ Low anti-CMV titer at month 1 increases the risk of CMV infection after heart transplantation (Figure 3). In a multivariate analysis of prospectively recorded data from 71 heart transplant patients (with CMVIG administered only in high-risk cases), 10 patients developed CMV disease.⁴⁸ Low anti-CMV titer either pretransplant (relative hazard, 8.1; $P = 0.004$) or at 1 month posttransplant (relative hazard, 4.49; $P = 0.02$) was found to be associated with a higher risk of CMV disease.⁴⁸ As might be expected, the combination of low anti-CMV IgG titer and low CD8⁺ CMV-specific T-cell count is particularly unfavorable in terms of risk for CMV infection³⁹ (Figure 4).

Cellular Response in Organ Transplant Recipients

Immediately after organ transplantation, there is a marked decline in numbers of CD3⁺, CD4⁺, and CD8⁺ T-cell counts.^{40,52} In a longitudinal immunological study of 88 kidney transplant patients, a short-term suppression of CMV-specific T cells was followed by normalization in the long term.^{30,52} This was accompanied by a decreased frequency of T_{reg} cells over the first few weeks posttransplant, a reduction that persisted thereafter, permitting recovery of effector T cells as an adaptive response to immunosuppression therapy to maintain pathogen control.

Figure 2 illustrates changes in T-cell counts over the first month after heart transplantation, demonstrating that T-cell reconstitution is significantly higher in those patients who remain free of infection. An analysis of the subpopulation of patients in that study who developed CMV disease ($n = 12$) showed that the absolute numbers of CD3⁺ T cells and CD8⁺ cytotoxic T cells during the first month posttransplant were significantly lower than in those without CMV infection.⁴⁰ Moreover, among patients with CMV infection the CD8⁺ T-cell count was significantly lower at month 1 in patients who progressed to CMV disease. Evidence from kidney transplantation has confirmed that CMV-specific CD4⁺ T-cell frequency shows an inverse correlation with the incidences of CMV replication, high CMV load, and onset of CMV disease.^{30,53,54} A study of 48 CMV-seropositive heart transplant patients, all treated preemptively with antiviral therapy, found a clear inverse association between immune recovery and risk of CMV viremia.⁵⁵ When the frequency of circulating CMV-specific CD4⁺ T cells falls below

approximately 0.25%, viral replication is no longer controlled.³⁰ In summary, transplant patients who maintain adequate, stable CMV-specific T-cell frequencies are less likely to develop CMV complications than those who over the first few months posttransplant have a sustained decrease in CMV-specific immunity.³⁰

In maintenance kidney and heart transplant patients, tapering of immunosuppressive doses can permit immune reconstitution to the point at which CMV-specific T-cell counts are similar to those in healthy controls,²³ such that late CMV reactivation is unlikely. However, low frequencies tend to persist in lung transplant patients,²³ a group at high risk for late-onset CMV viremia.⁵⁶

The Potential Immunomodulatory Effect of CMVIG

The CMVIG appears to influence the immune response via multiple mechanisms (Figure 1). There is evidence that it might affect some components of both innate and adaptive immune responses and exerts immunomodulatory activity (Table 1), but its complex mode of action has not been fully elucidated and further data are required.

Effect of CMVIG on Dendritic Cells

Data on whether and how CMVIG affects dendritic cell activity are lacking. One retrospective study has shown that in a series of 18 liver transplant recipients administration of CMVIG suppressed functional dendritic cell maturation⁵⁸ and alloantigen-stimulated T-cell proliferation. This is interesting because both components (dendritic cells and T cells) participate in the pathogenesis of allograft rejection. However, confirmatory data are lacking.

Effect of CMVIG on Humoral Immunity

Administration of CMVIG aims to restore normal concentrations of CMV-specific Ig in the immunocompromised patient. The CMVIG preparations are delivered as intact IgG molecules, which have a half-life similar to that of serum

IgG.⁵⁹ After administration of approximately 100 mg/kg body weight of a CMVIG to seronegative healthy subjects, enzyme-linked immunosorbent assay has shown an increase in specific CMV-IgG antibodies as well as a significant enhancement of virus neutralization *in vivo*.⁶⁰ The decline of both titers was biphasic: CMV IgG antibodies fell slowly during the first week and remained unchanged thereafter, whereas neutralization titers decreased markedly faster in the first than in the second week. Interestingly, in CMV-seropositive subjects, CMV IgG antibodies increased by approximately 3-fold, followed by a similar biphasic decline to that seen in seronegative subjects.⁶⁰

The CMVIG preparations are purified Ig products, obtained from pooled adult human plasma that has been selected for high anti-CMV antibody titers. A small number of studies have compared the titer of anti-CMV specific antibodies using CMVIG versus nonspecific intravenous immunoglobulin (IVIg) and found conflicting results.^{61,62} It seems that all commercially available IVIg products provide passive CMV neutralizing antibodies, but the level of circulating antibodies is affected by the IVIg product used.⁶³ It has been suggested that measurement of neutralizing activity against distinct cell lines and evaluation of other properties, such as avidity to CMV, are necessary when performing comparative studies.⁶⁴ It should also be borne in mind that different preparations are therefore not necessarily identical with regard to the titer of specific anti-CMV, and even within the same preparation differences may exist between different batches.

Replacement of IgG levels in heart recipients with severe and moderate hypogammaglobulinemia lead to lower rates of CMV infection in 2 studies performed at the Cleveland Clinic.^{51,65} One of these was a randomized clinical trial in which an intent to treat analysis showed a significant reduction of CMV infection in the CMVIG group compared with placebo (15.4% [2/13] vs 60% [6/10]; $P = 0.039$).⁶⁵ However, the patient numbers were small. We lack clinical trials comparing CMVIG versus IVIg in this indication.

TABLE 1.

Confirmed and possible immunomodulatory effects of CMVIG: an overview

Target	Mechanism/Proposed mechanism	Effect/Possible effect
Innate immunity cells Dendritic cells	Binds to Fc receptors	Possible suppression of functional dendritic cell maturation and alloantigen-stimulated T-cell proliferation
NK cells	Possible downregulation of cell-bound immunoglobulin and Fcγ RIII surface expression	May attenuate antibody-dependent cellular cytotoxicity effector functions
Adaptive immunity cells B cells	May increase levels of naïve B cells	
T cells	Inhibits IL-2, IFN-γ, and IL-10 production by T cells Decrease in CD4 ⁺ and CD8 ⁺ T-cell activation. CD8 ⁺ T-cell apoptosis	Inhibition of T-cell proliferation
Antibody levels	Passive transfer of antibodies	Increase in the level of specific anti-CMV neutralizing antibodies. Correction of Ig hypogammaglobulinemia Viral neutralization associated with high-avidity antibodies
CMV target cells	Prevention of CMV binding to target cells Neutralizing antibody response to cell surface-expressed CMV antigens gH/gL/UL128/UL130/UL131 complex ⁵⁷	Modulation of CMV infection and disease

The importance of passive transfer of antibody by CMVIG seems to be greatest during the initial phase of infection. The kinetics of specific anti-CMV antibodies in seronegative recipients after introduction of CMVIG has not previously been reported in heart transplantation. In a series of 6 high-risk (D+/R-) heart transplant patients in whom 8 doses of CMVIG were administered between days 0 and 77 (150 mg/kg within 24 hours after transplantation and 100 mg/kg on days 2, 7, 14, 22, 35, 56, and 77), there was a significant increase in anti-CMV antibody titer from pretransplant level and day 7.⁴⁸ Titers remained above baseline to the end of the 1-year study but decreased after CMVIG was stopped at 70 days.⁴⁸ Anti-CMVIG levels remained significantly lower than in a control group of non-high-risk patients (seropositive recipients with prior CMV infection) without CMV infection after transplantation. Four of the 6 CMV-seronegative patients developed CMV disease. The CMV disease developed significantly later in these patients compared with D+R+ patients evaluated in the same study (5 ± 14 vs 2 ± 0.5 months; $P = 0.003$). These observations suggest that CMVIG might provide early protection while titers are high. However, this analysis was performed in a small number of patients and should be further evaluated in future studies, including testing for neutralizing antibodies.⁶⁶

Effect of CMVIG on Anti-CMV Cellular Immune Response

Data remain limited regarding the influence of CMVIG administration on the cellular immune cascade. In vitro data have shown that application of CMVIG decreases production of the cytokines IL-2, IFN- γ , and IL-10 in mixed lymphocyte reactions and anti-CD3 blastogenesis assays.⁶⁷ In the same study, CMVIG was found to induce apoptosis of CD8⁺ T cells and NK cells and attenuate antibody-dependent effector cell cytotoxicity functions.⁶⁷ Another group has also reported that CMVIG suppresses cytokine production in vitro and inhibits T-cell proliferation.⁶⁸ A comparative in vitro study found no difference in the type of inhibitory effect between IVIg and CMVIG, but IVIg resulted in a lower degree of inhibition ($P = 0.05$).⁶⁹

Future studies should evaluate whether the immunomodulatory effect of CMVIG on T-cell activation is associated with lower rates of allograft rejection. After heart⁶⁵ or lung⁷⁰ transplantation, use of CMVIG has been associated with lower rates of acute rejection but the evidence is by no means clear-cut,⁷¹ and this remains unconfirmed in other types of solid organ transplantation. The CMVIG impacted positively on the rate of acute rejection in liver recipients in a study performed by Farges et al,⁷² whereas another study performed by Kwekkeboom et al⁵⁸ failed to find a significantly lower rate of acute rejection among seronegative liver patients treated with CMVIG.

CONCLUSIONS

The immune response to CMV is highly complex, involving multiple components of the immunological cascade. As a result, it is unlikely that any single diagnostic test or therapeutic intervention is enough to deliver effective management in thoracic transplant recipients. A combination of different diagnostic and therapeutic interventions would appear to offer a better strategy.

Passive transfer of specific anti-CMV antibodies from CMVIG appears to be accompanied by some degree of immune modulation. These could play a role in suppressing some of the direct and indirect effects of CMV infection. The immunomodulatory effects of CMVIG could also, potentially, improve control of other transplant-related complications, such as allograft rejection, but this remains unconfirmed.

Further studies are required to assess the potential immunomodulatory impact of CMVIG on distinct components of the immune response to CMV, including antigen presentation (macrophages, dendritic cells), effector cells (NK cells, CD4⁺, CD8⁺, and CD19⁺ specific cells) and to determine whether this is qualitatively different from that of IVIg preparations. Additional work is also needed to confirm that anti-CMV specific antibody titers, avidity and neutralizing activity are higher using CMVIG compared with IVIg.

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