

State-of-the-Art Monitoring of Cytomegalovirus-Specific Cell-Mediated Immunity After Organ Transplant: A Primer for the Clinician

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Cytomegalovirus (CMV) is one of the most common infections after solid organ transplantation. Improved assays to predict viral replication and disease would help refine current preventive strategies. Monitoring of CMV-specific T-cell responses may help guide clinical decision making. Several techniques are now available to quantify CMV-specific T-cell responses, including flow cytometry, enzyme-linked immunosorbent spot assay, and enzyme-linked immunosorbent assay. Standardization and validation of these assays have the potential to significantly change the monitoring and treatment of CMV and further personalize CMV prevention strategies. In this review, we discuss the measurement of CMV-specific T-cell responses and their clinical impact on the management of CMV after organ transplantation.

One of the most important viral pathogens in transplant recipients is cytomegalovirus (CMV). CMV may cause significant morbidity and occasional mortality and may have deleterious effects on graft function [1, 2]. The host-virus interaction that ultimately leads to CMV replication posttransplant is complex and involves the interplay of the innate and adaptive immune systems, the infected tissue, and virus-mediated immune modulation. Following transplantation, induction treatment with T-cell-depleting antibodies, prolonged immunosuppression, the human leukocyte antigen (HLA) mismatch between graft and host immune cells all combine to disrupt the balance between viral replication and control. In some patients this leads to progressive viral replication, end-organ disease, and impaired long-term graft outcome [1].

After organ transplantation, patients at high risk for CMV disease (eg, donor seropositive, recipient seronegative [D⁺/R⁻]) generally receive antiviral prophylaxis. Intermediate-risk patients (eg, recipient seropositive [R⁺]) may either receive prophylaxis or may be monitored using molecular detection techniques with preemptive therapy started at a predefined cutoff value or increasing viral load. However, despite these measures, viremia and disease remain common in certain settings. For example, 30%–40% of D⁺/R⁻ patients will develop CMV disease after discontinuation of 3 months of prophylaxis [3]. In patients on preemptive strategy approaches, viral load thresholds for initiating treatment are not well defined, and are hampered by the fact that many patients with low-level CMV viremia will have spontaneous clearance without the need for antiviral therapy. Finally, patients with a previously treated episode of CMV disease may undergo virologic and clinical relapse [4]. In summary, current prediction algorithms that rely on pretransplant serostatus and posttransplant viral load testing are suboptimal and only weakly correlate with the risk of CMV disease in certain settings [5, 6]. Therefore, new predictive biomarkers would have important clinical utility in efforts to prevent and treat CMV disease. In the past 2 decades, it has become clear that CMV-

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specific immunity plays a critical role in the development and severity of CMV disease [7–11]. Therefore, the analysis of CMV-specific T-cell frequencies and function can potentially allow direct quantification of the patient's ability to control CMV. With these methods, several outstanding questions can be addressed, including (1) the risk of late-onset CMV viremia and disease following antiviral prophylaxis; (2) the risk of progression to disease vs spontaneous viral clearance in patients with low-level viremia; and (3) the risk of recurrent viremia and disease following a course of treatment. In this review, we discuss the principles of CMV-specific T-cell monitoring and its current role in the clinical management of organ transplant recipients.

INNATE AND ADAPTIVE IMMUNITY

In the posttransplant setting, both innate and adaptive immune responses play a role in the control of viral pathogens. The importance of innate immunity in CMV control is only partially understood. Studies evaluating single-nucleotide polymorphisms (SNPs) in innate immune genes have suggested links between certain SNPs and the risk of CMV reactivation. For example, transplant recipients with an SNP in Toll-like receptor 2 (TLR2, R753Q) and in the promoter of the dendritic cell-specific ICAM3-grabbing nonintegrin (DC-SIGN) showed an increased risk of both prolonged CMV replication and disease [12, 13]. Additionally, the expression of activating killer-cell immunoglobulin-like receptors on natural killer cells has been negatively correlated with CMV replication [14]. It is likely that interactions between several arms of the innate immune system contribute to CMV control, and further elucidation of these pathways may provide more information and potential biomarkers for future clinical use.

The crucial role of adaptive immunity, such as neutralizing antibodies and virus-specific CD4^{pos} and CD8^{pos} T-cell responses, has been more conclusively established. CMV-specific neutralizing antibodies appear 2–4 weeks after primary infection and are mainly directed against CMV glycoprotein B, H, L, and pUL128–131 [15]. Neutralizing antibodies are generated in response to CMV infection; measurement of humoral immune responses (CMV serology) as a predictive tool is primarily limited to pretransplant assessment. Prior to transplant, CMV serology of donor and recipient is commonly used to stratify for risk of CMV replication and disease posttransplant. CMV-seronegative recipients (R[−]) of seropositive grafts (D⁺) are at highest risk of CMV disease. Although pretransplant serology is routinely used to inform decisions regarding optimal posttransplant prevention strategies, posttransplant seroconversion is not a reliable predictor of CMV disease [6].

T-CELL ACTIVATION

Priming of T cells requires a number of interactions between the antigen-presenting cell (APC) and the epitope-specific T cell (Figure 1). APCs present, in an HLA-dependent context, epitopes via the major histocompatibility complex (MHC) I or II to the T-cell receptor (Signal 1). In addition, T-cell activation is dependent on the interaction of costimulatory receptors on T cells and APCs. This interaction further modulates the immune response (Signal 2) [16]. Persistent virus replication is associated with an upregulation of inhibitory receptors such as PD-1 (programmed death receptor), T-cell immunoglobulin domain and mucin domain 3, and cytotoxic T-lymphocyte antigen 4. This causes a loss of function and is commonly known as T-cell exhaustion [17].

A highly diverse virus-specific T-cell response develops between 4 and 6 weeks after primary antigen exposure. The memory compartment is generated, based upon the amount of antigen, the replication pattern and the type of infected tissue. T-cell memory subsets generated are phenotypically defined by their surface marker profile. For example, virus replication without antigen clearance (eg, chronic CMV infection) generates a CD27^{neg} CD28^{neg} CD45RA^{pos} CCR7^{neg} T-cell memory compartment [18].

Normal T-cell function can be inhibited by coreceptors. One inhibitory coreceptor of recent interest is PD-1. PD-1 is a coreceptor on T cells, associated with an inhibition of virus-specific T-cell proliferation and function in chronic infections such as human immunodeficiency virus and hepatitis C virus [16]. PD-1 expression on CMV-specific CD4^{pos} T-cells of R⁺ patients was associated with reduced interleukin 2 (IL-2) production and inhibition of proliferation. This effect was reversible upon treating with PD-1-blocking antibodies [17]. Other inhibitory biomarkers have also been described. An inverse association between plasma interleukin 10 and CMV-specific T-cell responses has been reported [19]. Other T-cell subtypes such as total Th17 and regulatory T-cells have not been well studied in organ transplant patients with CMV reactivation.

ASSAYS TO MEASURE CMV-SPECIFIC T-CELL RESPONSES

General Principles and Controls

A variety of assays exist to measure CMV-specific cellular responses. The basic principle of most assays is the CMV-specific stimulation of T cells for 6–18 hours in cell culture. Following stimulation, the T cells are either fixed or stained with antibodies, for example, against intracellular interferon γ (IFN- γ) or other cytokines; or the supernatant is used to measure cytokine release. Other cytokines such as tumor necrosis factor α or IL-2 could also be measured, although

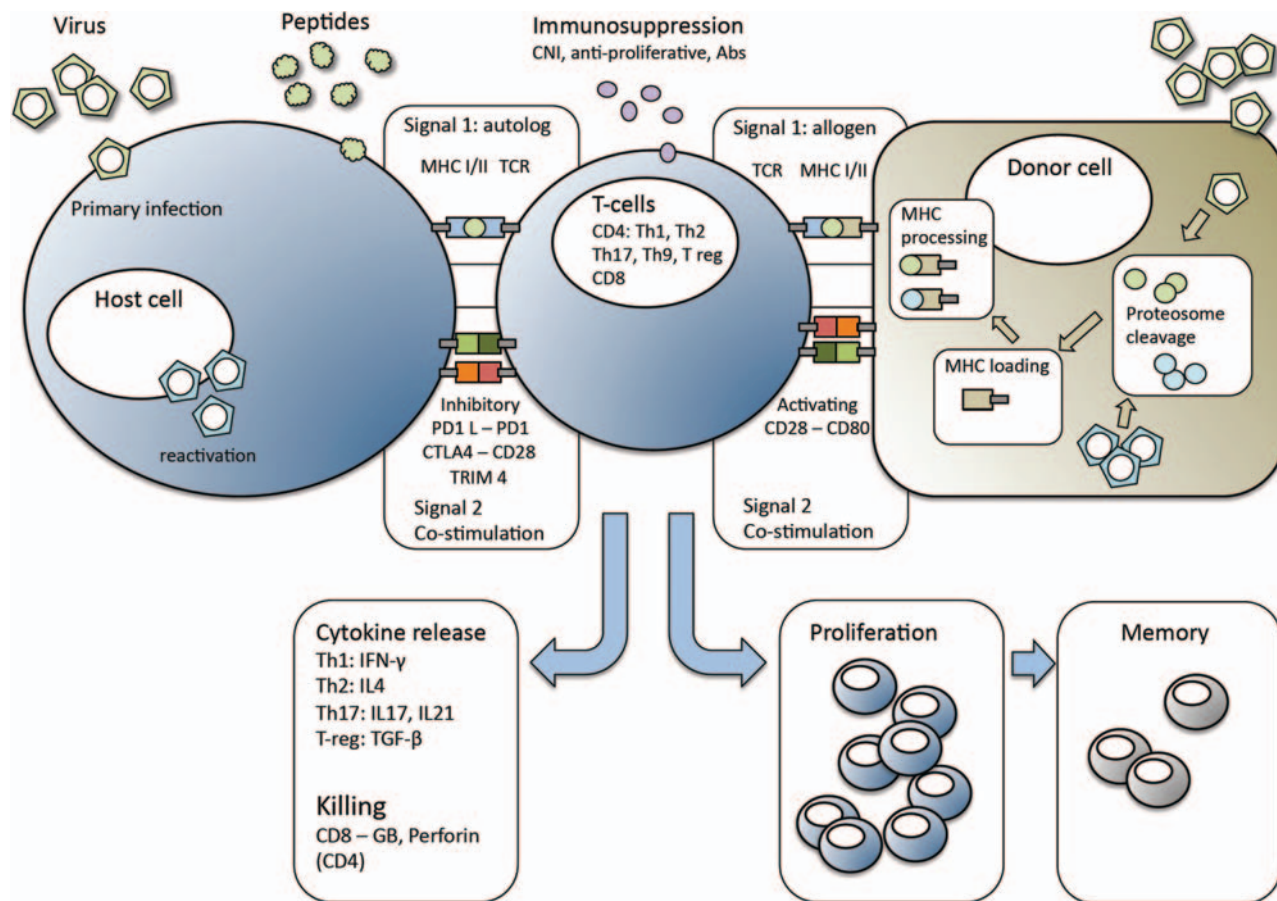


Figure 1. Interaction of cytomegalovirus (CMV)-infected cell or antigen presenting cell with a CMV-specific T cell. Viral proteins are processed by both host cell and donor cell. Viral epitopes are then presented via major histocompatibility complex (MHC) I or II on an antigen-presenting cell and are recognized by a corresponding virus-specific T cell (Signal 1). Signal 1 can occur in an autologous or allogeneic context. The second signal is binding of costimulatory receptors and ligands for either activation (eg, CD28–CD80/86) or inhibition (CTLA4–CD28). Based on immunosuppression, differing amounts of cytokines (eg, Th1 and Th2 cytokines) are released and T-cell proliferation occurs to build and establish immunological memory. Abbreviations: Abs, antibody therapy; CNI, calcineurin inhibitor; GB, granzyme B; IL, interleukin; TCR, T-cell receptor; TGF, transforming growth factor.

IFN- γ is the best documented in the literature. Figure 2 outlines commonly used techniques: flow cytometry using intracellular cytokine and tetramer staining, enzyme-linked immunosorbent (ELISA)-based assays, enzyme-linked immunosorbent spot assay (ELISpot), cytokine profiling, and T-cell proliferation assays. Table 1 provides an overview of the laboratory requirements, and the advantages and disadvantages of each individual method. A Food and Drug Administration (FDA)-approved, adenosine triphosphate release assay (Immuknow, Cylex Inc) is used in several centers as a global measure of immunosuppression; however, it is important to note that although low values obtained with this assay may be predictive of infections in general, the assay is not pathogen specific [20]. The majority of CMV-specific assays have been used in research to determine their predictive value in CMV

viremia or disease. No CMV-specific assay is FDA approved; however, an ELISA-based assay (Quantiferon-CMV, Cellestis Inc) has been commercialized and approved in the European Union as conforming to regulatory requirements.

For quality-control purposes, immune assays generally contain controls. The positive control (eg, phytohemagglutinin or staphylococcal enterotoxin B) helps to identify patients whose T cells are unresponsive. This is most likely due to potent immunosuppression, preanalytic errors, or very low lymphocyte frequencies. Virus-specific results obtained from patient samples that have a low or undetectable positive control value may be difficult to interpret. For example, in a study of 108 posttransplant patients, almost 30% of Quantiferon-CMV assays showed no response to mitogen stimulation [21]. The negative control (eg, cell media or mock antigen)

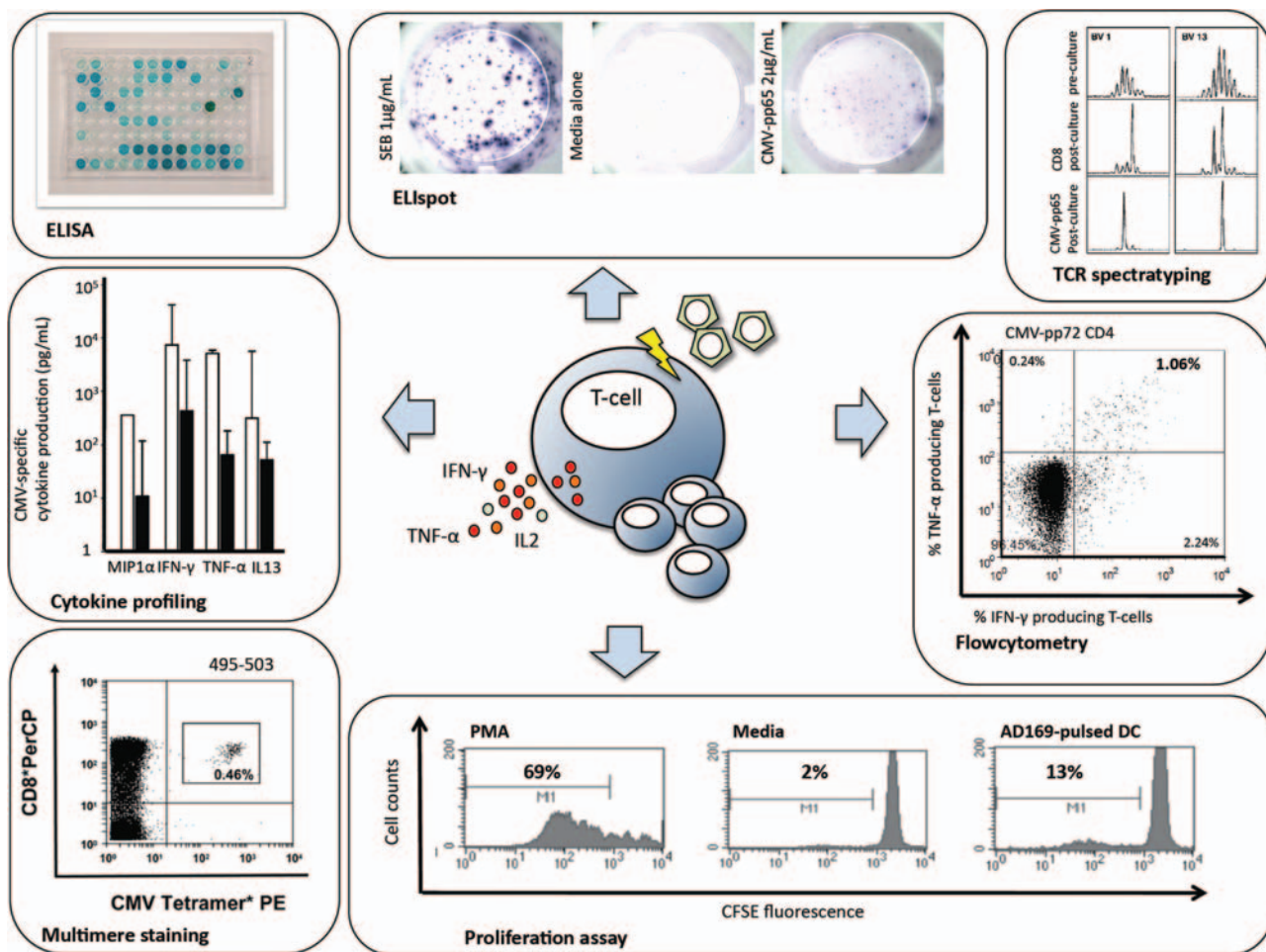


Figure 2. Methods of measuring cytomegalovirus (CMV)-specific T cells. Current methods to determine CMV-specific T-cell functions include flow cytometry, enzyme-linked immunosorbent spot assay, enzyme-linked immunosorbent assay, cytokine profiling, tetramer staining, T cell receptor spectratyping, and expansion protocols. Abbreviations: CFSE, carboxyfluorescein diacetate succinimidyl ester; CMV, cytomegalovirus; DC, dendritic cell; ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunosorbent spot assay; IFN, interferon; IL, interleukin; PE, phycoerythrin; PMA, phorbol 12-myristate 12-acetate; SEB, staphylococcal enterotoxin B; TCR, T cell receptor; TNF, tumor necrosis factor.

identifies patients who show nonspecific background reactivity. The majority of currently existing assays have no well-validated cutoff for defining positivity. An exception to this is the Quantiferon-CMV assay, for which a positive value is defined as an IFN-γ level ≥ 0.2 IU/mL, although this has not been well validated in the transplant population. Measurements with cytokine flow cytometry can allow detailed analysis of percentage of T cells and T-cell subsets required to confer protection from CMV. However, these have not been standardized and the frequency of T cells that allow for protection from CMV is unclear. Protective levels have ranged from 0.03% to 2% [8, 11]. No established cutoffs are developed for ELISPOT assays. Different thresholds for a positive test may exist depending on the immune assay used, patient group studied, and the clinical setting. Therefore,

comparison between methods is difficult due to lack of standardization.

Cell Stimulation

In vitro CMV-specific immune response can be induced using single peptides, peptide libraries, or whole virus lysate. The amino acid sequence and length of the peptide significantly influences the type of immune response that is induced. An “immunodominant” peptide contains epitopes able to stimulate T cells with a specific HLA background. A disadvantage of using single peptides is that they might exclude certain HLA types and the test may potentially exhibit no stimulation. The Quantiferon-CMV assay contains a combination of 22 peptides for cell stimulation. However, patients with uncommon HLA types may be negative by this assay. Peptide

Table 1. Overview of Techniques Used to Measure Cytomegalovirus-Specific T-Cell Responses

	ATP Release Assay ^a	Quantiferon-CMV ^b	Cytokine Profiling (Bead-Based Technology)	ELISpot Assay	Intracellular Cytokine Staining (Flow Cytometry)	Tetramer Staining (Flow Cytometry)
Material	Whole blood, then CD4 ^{pos} T-cell separation with magnetic beads	Whole blood	Whole blood or PBMCs	PBMCs	Whole blood or PBMCs	PBMCs
Quantity	1–3 mL	3 mL	1 mL; 0.25–1 × 10 ⁶ cells	0.25 × 10 ⁶ cells	0.5–1 mL; 0.25–1 × 10 ⁶ cells	0.25–1 × 10 ⁶ cells
Time	48 h	24 h	48–72 h	48 h	48 h	48 h
Antigen	PHA	Peptides, mitogen	Peptides, lysate, mitogen	Peptides, lysate, mitogen	Peptides, lysate, mitogen	Peptides, lysate, mitogen
Frequency	Nonspecific	CMV specific	CMV specific	SFU/mL	CMV-specific T cells	Epitope specific T cells
Function	ATP release	IFN-γ IU/mL	Cytokine release panels for a variety of cytokines	Variety of cytokines (eg. IFN-γ)		Frequency of T-cell phenotypes; need to combine with ICS for function
Advantages	Commercial test FDA approved	Highly sensitive CE test	High sensitivity Several cytokines from minimal sample amount	High sensitivity	Potential to measure a variety of phenotypic-, regulatory- and memory-cell markers, coreceptors and cytokines	Single epitope-specific clone staining
Disadvantages	No CMV-specific response	Quantiferon-CMV assay is a CD8 ^{pos} T-cell assay	Research tool Multiplex reader Large amount of data requiring special analysis	No differentiation between CD4 ^{pos} and CD8 ^{pos} T cells ELISpot reader required	Labor intensive Flow cytometer required Research tool	Similar to ICS Knowledge on individual HLA-type required

Abbreviations: ATP, adenosine triphosphate; CE, Conformité Européenne; CMV, cytomegalovirus; ELISpot, enzyme-linked immunosorbent spot assay; FDA, Food and Drug Administration; HLA, human leukocyte antigen; ICS, intracellular cytokine staining; IFN, interferon; PBMCs, peripheral blood mononuclear cells; PHA, phytohemagglutinin; SFU, spot-forming unit.

^a Cylex Immuknow assay.

^b Quantiferon-CMV (Cellestis Inc) is an enzyme-linked immunosorbent-based assay. The assay is considered to stimulate primarily a CMV-specific CD8^{pos} T-cell response based on peptide length included in the CMV antigen tube.

libraries used for stimulation, covering 1 protein with 15 amino acid–long sequences with an 11 amino acid overlap, are specific, but potentially less sensitive; whole virus lysate may be more sensitive due to expression of several CMV proteins; however, lysate is generated from infected fibroblasts and can have variability between batches and is therefore difficult to standardize.

The frequency of epitope-specific T cells varies for different viral proteins. In one study, 151 CMV open reading frames (ORFs) were immunogenic for CD4^{pos} and/or CD8^{pos} T cells, sharing 8 of 15 top recognized ORFs [22]. Skewing of the T-cell receptor β variable repertoire during replication indicates that the dominant antigens vary during virus replication [23]. Therefore, the virus-specific immune response alters during the course of replication depending on the dominant antigen. Thus, for clinical purposes, an immune monitoring assay should include a spectrum of viral proteins to reflect this variability.

CLINICAL APPLICATION OF CMV-SPECIFIC T-CELL RESPONSE AFTER TRANSPLANTATION

The risk of CMV replication posttransplant is dependent on several factors including pretransplant serostatus of donor and recipients (highest risk is D⁺/R⁻), type of transplant, time posttransplantation, immunosuppressive regimen, usage of T-cell-depleting antibodies, age, sex, HLA type, and HLA mismatch [1, 2]. Given the limitations in accurately predicting who will be at risk for CMV posttransplant, measurements of CMV-specific CD4^{pos} and CD8^{pos} T cells can potentially individualize prophylaxis and preemptive strategies. Initial attempts to correlate CMV replication and CMV-specific T-cell responses were conducted in hematopoietic stem cell transplant (HSCT) recipients [24]. Subsequently, similar studies in solid organ transplantation showed that recipients with sufficient CMV-specific CD4^{pos} and/or CD8^{pos} T-cell responses were protected from CMV replication and progression to disease [7, 8]. Figure 3 illustrates the immunological response in 2 different clinical scenarios: progressive replication vs spontaneous clearance. Specific clinical uses of CMV-specific cell-mediated immunity (CMI) assays are discussed below.

Prediction of Postprophylaxis CMV in High-Risk Patients

Antiviral prophylaxis is commonly given to D⁺/R⁻ patients for 3–6 months based on several randomized trials showing a benefit to this approach [3, 25]. However, the risk of viremia and disease occurring after the discontinuation of prophylaxis (termed late-onset CMV) remains significant. Recent studies identified that late-onset CMV disease can be reduced by prolonging antiviral prophylaxis to 6 months posttransplant [26, 27]. Prolonged prophylaxis may inhibit the development of CMV-specific T-cell immunity. This has been shown in the

HSCT population where ganciclovir prophylaxis inhibits the development of CMV-specific immunity owing to either the prevention of priming or direct T-cell inhibition [28]. However, during CMV replication, D⁺/R⁻ patients have an initial CMV-specific CD8^{pos} T-cell response, but after antiviral therapy, a CMV-specific CD4^{pos} T-cell response predominates [29].

CMV-specific T-cell responses have been studied as clinical markers in this population in an attempt to predict CMV viremia and disease. However, to date, these data have been conflicting and limited by small samples sizes of studies (Table 2). For example, 17 D⁺/R⁻ liver transplant recipients were followed with serial immune monitoring up to 12 months posttransplant. Although one-third of patients developed symptomatic disease, CMV-specific CD4^{pos} and CD8^{pos} T-cell responses were not predictive of disease development [10]. Similarly, 22 high-risk lung transplants who became viremic were followed for relapse of disease. CD8^{pos} T-cell frequencies during primary viremia in this cohort were not associated with relapsing viremia [30]. In a larger study of 108 transplant patients (D⁺/R⁻ and selected R⁺ at high risk), measurement of IFN- γ by the Quantiferon-CMV assay at the completion of antiviral prophylaxis was predictive of CMV disease occurring after prophylaxis. However, the D⁺/R⁻ subset only comprised 32.4% patients in this study [21]. A larger multicenter study of D⁺/R⁻ patients has recently been completed and may help determine if Quantiferon-CMV assays can be used to predict late onset disease following prophylaxis.

Prediction of CMV Replication in Seropositive (R⁺) Patients

CMI assays could be used in this population to refine preemptive protocols or help guide duration of prophylaxis. In contrast to D⁺/R⁻ patients, who need to prime an initial CMV-specific T-cell response, R⁺ transplant patients already have established immunological memory. In CMV-seropositive patients, immune reconstitution of CMV-specific memory T cells is a crucial predictor of subsequent CMV reactivation. Early reconstitution (within 1 month) of CMV-specific T cells tends to be associated with asymptomatic viremia or mild disease vs delayed reconstitution [9, 31]. Interestingly, after 6 months, CMV-specific CD4^{pos} T cells seem to be more important in controlling CMV replication. For example, patients with asymptomatic CMV viremia have a prominent CMV-specific CD4^{pos} T-cell response compared with symptomatic patients [31].

Several investigators have studied the predictive value of CMI assays in the R⁺ setting. The majority of studies use cytokine flow cytometry and stimulation of cells with a variety of CMV antigens. Most show a relationship between poor virus-specific immune response and CMV viremia. However, due to a large variability in responses, a predictive cutoff value has been difficult to establish. In one study of 27 heart and lung transplant patients, high frequencies of IE-1-specific CD8^{pos}

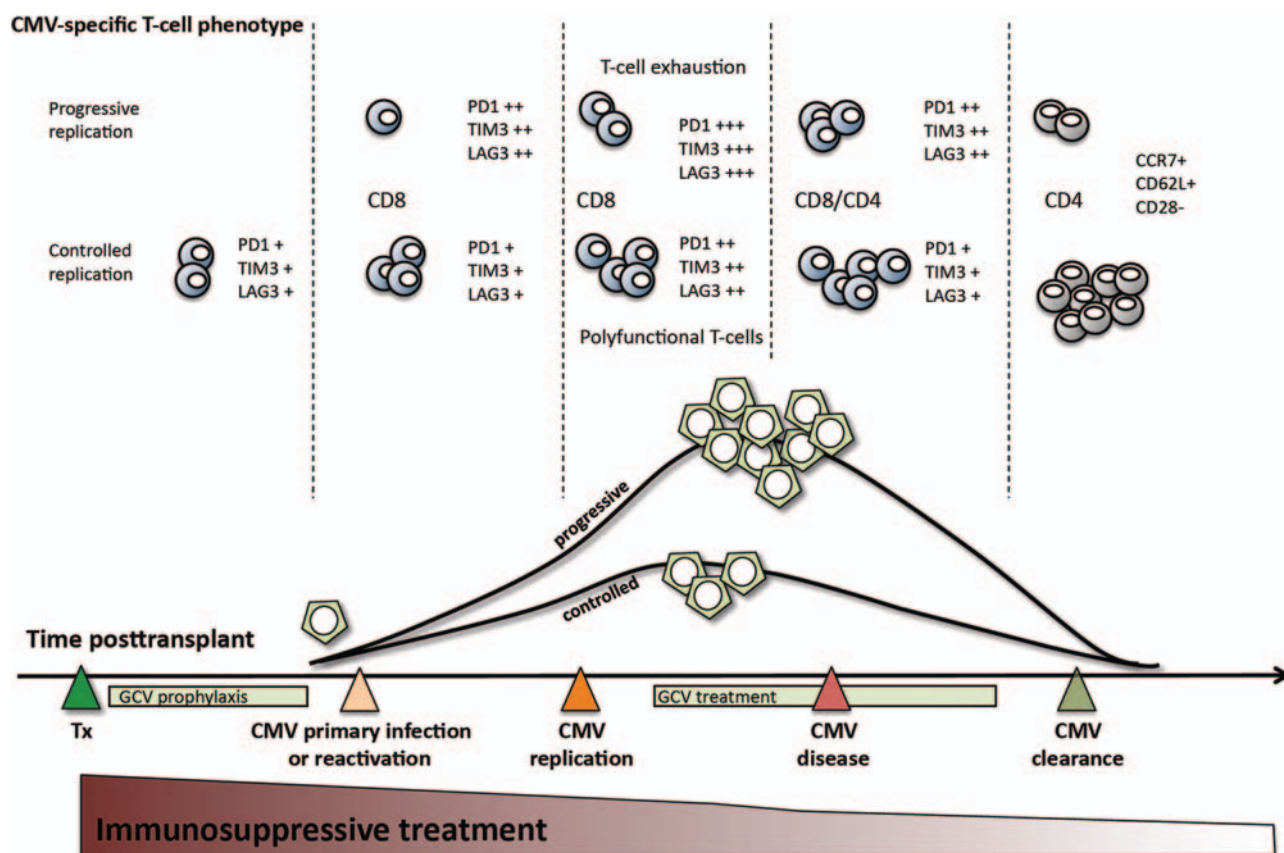


Figure 3. Cytomegalovirus (CMV)-specific T-cell and CMV replication—a dynamic process. Posttransplant, viral replication may occur in a progressive or controlled manner. Different CMV-specific phenotypes occur depending on viral replication pattern. For example, in progressive replication, high expression of inhibitory costimulatory signals is observed (eg. PD-1) resulting in reduced T-cell proliferation (exhausted phenotype). In controlled replication, polyfunctional T cells predominate. In both phenotypes, initially a CD8 response occurs, followed by establishment of a CD4 response as viral clearance occurs. Abbreviations: CMV, cytomegalovirus; GCV, ganciclovir; LAG, lymphocyte-activation gene; PD, programmed death receptor; TIM, T-cell immunoglobulin domain and mucin domain.

T-cells $>0.4\%$ at any time after transplant were protective against CMV disease [7]. Abate et al have used the ELISpot assay method to show that patients who develop CMV viremia have significantly lower IFN- γ expression prior to viremia than those without viremia [32, 33]. In these studies, low responders were characterized as having <50 spot-forming units (SFU) per 200 000 cells. The Quantiferon-CMV assay, primarily characterizing CD8^{pos} T-cell function, has been used in several studies of R⁺ patients [21, 34–36]. In a lung transplant cohort, a single measurement with this assay was not predictive of CMV reactivation as measured by viral loads in bronchoalveolar lavage fluid [36]; however, in a cohort of kidney transplant patients and a cohort of R⁺ transplant patients at high risk of CMV disease, Quantiferon-CMV assay results were able to predict subsequent CMV disease [21, 35]. Another study recently showed that this assay, when performed at the onset of viremia, was able to differentiate

whether patients would spontaneously resolve viremia or require antiviral treatment [37].

Clinical Prediction of Treatment Response and Relapse

CMV-specific T-cell immunity could potentially be used in other clinical situations. For example, CMV-specific T-cell function could be used to predict the risk of relapse after an episode of CMV disease (Table 3). Patients with no T-cell response at the end of therapy may benefit from secondary prophylaxis or more intensive monitoring. Conversely, sufficient CMV-specific T-cell control in a patient on therapy for CMV could enable timely discontinuation of antiviral therapy and reduce the need for intensive monitoring. It is important to note that immunologic monitoring is not meant to replace viral load testing but should be used as an adjunct method to predict persons at risk for viremia and disease.

Table 2. Studies of Cytomegalovirus-Specific Immune Monitoring in Organ Transplant Patients to Predict Cytomegalovirus Viremia or Disease

Reference	Population	Monitoring Frequency	CMV Serostatus	Assay	Findings
Sester M et al, 2001 [38]	N = 76 kidney 42 CMV ⁺ controls 66 CMV ⁻ controls	Cross-sectional analysis at pre-tx and 2, 6, 12 mo post-tx	R ⁺ (100%)	CFC assay after stimulation with viral lysate	CD4 ⁺ T-cell frequencies inversely correlate with CMV viral load
Bunde et al, 2005 [7]	N = 27 heart and lung	Monitoring weekly to monthly up to 2 y post-tx	R ⁺ (100%)	CFC using pp65 and IE-1 peptide pools	IE-1 (but not pp65)-specific CD8 ⁺ T-cell frequency >0.4% at 2 wk post-tx predict disease
Sester U et al, 2005 [11]	N = 96 SOT N = 50 controls	Single timepoint at >6 mo post-tx	R ⁺ (100%)	CFC assay after stimulation with viral lysate	Low levels of CMV-specific CD4 correlates with CMV viremia Lung tx patients had the lowest T-cell responses
Radha et al, 2005 [39]	N = 33 kidney; 17 controls; 6 active CMV viremia	Assay done at variable times post-tx	R ⁺ (73%) D ⁺ /R ⁻ (9%) D ⁻ /R ⁻ (18%)	CFC using viral lysate and pp65 peptide pool	CD8 ⁺ T-cell responses highly correlated with serostatus in tx and controls
Gerna et al, 2006 [9]	N = 38 SOT	Monthly till 6 mo post-tx, then at months 9 and 12	R ⁺ (100%)	CFC assay after stimulation with CMV-infected immature dendritic cells	Patients with early CMI (with 1 m of tx) had spontaneous resolution of viremia vs late development of CMI who required antiviral therapy; a cutoff of 0.4 cells/ μ L was predictive of protection from viremia
La Rosa et al, 2007 [10]	N = 17 liver and kidney	Monitoring every 2 wk from 3–6 mo post-tx	D ⁺ /R ⁻ (100%)	CFC assay after stimulation with viral lysate, pp65 and IE-1 peptide pools	Responses not predictive of viremia
Egli et al, 2008 [8]	N = 73 kidney	Every 2 wk till 4 mo post-tx, then monthly till 6 mo	R ⁺ 66% D ⁺ /R ⁻ 34%	CFC using viral lysate, pp72 and pp65 peptide pools	pp65 CD4 ⁺ and CD8 ⁺ responses were lower in patients with viremia; CD4 ⁺ IFN- γ response >0.03% correlated with absence of CMV
Westall et al, 2008 [36]	N = 39 lung	Samples at 0.5, 1, 2, 3, 6, 9, 12, 18 mo post-tx	R ⁺ (62%) D ⁺ /R ⁻ (21%) D ⁻ /R ⁻ (18%)	Quantiferon-CMV	Level of IFN- γ did not predict CMV reactivation as measured by BAL viral loads; in 5 patients that had significant CMV viral loads on BAL, 3/5 had a decrease in immune response prior to episode
Kumar et al, 2009 [21]	N = 108 SOT	Monthly monitoring for 4 mo after finishing prophylaxis	R ⁺ (68%) D ⁺ /R ⁻ (32%)	Quantiferon-CMV	Patients with detectable IFN- γ response had lower risk of CMV disease ($P = .038$)
Abate et al, 2010 [33]	N = 85 kidney; 27 pretransplant	Monitored pre-tx and 1, 2, 3, 6, 12 mo post-tx	R ⁺ (82%) D ⁺ /R ⁻ (15%) D ⁻ /R ⁻ (2%)	ELISpot to measure IFN- γ ⁺ T cells after stimulation with pp65 peptide pool	Patients with CMV viremia had significantly lower IFN- γ expression in the 2 mo prior to episode than those with no viremia ($P = .0003$); having viremia resulted in a significant IFN- γ response
Eid et al, 2010 [40]	N = 44 kidney	Samples at week 2, during 1–3 mo, 4–6 mo, and month 12	R ⁺ (75%) D ⁺ /R ⁻ (25%)	CFC assay after stimulation with viral lysate, pp65 and IE-1 peptide pools	Only 1 CMV viremia episode in R ⁺ patients; no association of CD4 and CD8 responses in D ⁺ /R ⁻ patients

Table 2 continued.

Reference	Population	Monitoring Frequency	CMV Serostatus	Assay	Findings
Chiereghin et al, 2010 [31]	N = 10 bowel/multivisceral	Monitoring monthly from time of transplant	R ⁺ (100%)	ELISpot to measure IFN- γ ⁺ T cells after stimulation with pp65 and IE-1 peptide pool	Lower viral loads were seen in those who developed T-cell responses early vs late responders who developed CMV disease
Sund et al, 2010 [41]	N = 17 kidney	Patients monitored for monthly for 1 y post-tx	R ⁺ (100%)	MHC tetramer loaded with pp65 peptides	Proportion of CD4 ⁺ IFN- γ ⁺ T cells at 2 mo post-tx correlated with the magnitude of CMV viral load
Pipeling et al, 2011 [30]	N = 22 lung	Before discontinuation of prophylaxis and within 2 wk of viremia	D ⁺ /R ⁻ (100%)	CFC using pp65 and IE-1 peptides	Patients with relapsing viremia had lower frequencies of pp65-associated CD4 and CD8 responses during primary infection
Costa et al, 2011 [42]	N = 24 lung	Monitored at 2 time points >1 y post-tx	R ⁺ (88%) D ⁺ /R ⁻ (4%) D ⁻ /R ⁻ (8%)	ELISpot to measure IFN- γ ⁺ T cells after stimulation with CMV peptides	Responders by ELISpot (>5 SFU/200 000 cells) had lower CMV viral loads on BAL than nonresponders ($P = .02$)
Gerna et al, 2011 [43]	N = 134 SOT	Monthly till 6 mo post-tx, then at months 9 and 12	R ⁺ (87%) R ⁻ (13%)	CFC assay after stimulation with CMV-infected immature dendritic cells	Having both CD4 and CD8 cells >0.4/ μ L is protective against CMV disease
Abate et al, 2012 [32]	N = 48 heart	At less than or greater than 100 d post-tx	R ⁺ (100%)	ELISpot to measure IFN- γ ⁺ T cells after stimulation with pp65 peptide pool	Patients with CMV viremia were low responders (<5 SFU/200 000 cells) in the 2 mo prior to episode than those with no viremia ($P < .05$); inverse correlation between viremia level and immune recovery
Lisboa et al, 2012 [37]	N = 37 SOT	All patients with low level CMV viremia monitoring at onset of viremia	R ⁺ (81%) D ⁺ /R ⁻ (19%)	Quantiferon-CMV	Spontaneous viral clearance in those with positive test vs progression to treatment with negative test ($P = .004$)
Weseslindtner et al, 2012 [44]	N = 67 lung	Monitored for 1 y post-tx with mean monitoring interval 26 d	R ⁺ (58.2%) D ⁺ /R ⁻ (25.4%) D ⁻ /R ⁻ (n = 16.4%)	Quantiferon-CMV	A negative CMI was associated with a greater proportion of patients who had CMV viral loads >1000 copies/mL than those with positive CMI ($P = .0046$) serial measurements are needed to detect short-term fluctuations in CMI that may be associated with CMV disease
Patel et al, 2012 ^a [45]	N = 9 pediatric SOT & 1 stem cell tx; 8 controls; 14 children >1 y post-tx	Monitored at 1, 3, 6 mo post-tx (n = 10)	R ⁺ (60%) D ⁺ /R ⁻ (10%) D ⁻ /R ⁻ (30%)	ELISpot to measure IFN- γ ⁺ T cells after stimulation with pp65 peptide pool	Very few patients to conclude the effectiveness of monitoring but study demonstrates feasibility of monitoring in pediatrics

Abbreviations: BAL, bronchoalveolar lavage; CFC, cytokine flow cytometry; CMI, cell-mediated immunity; CMV, cytomegalovirus; ELISpot, enzyme-linked immunosorbent assay; IE, immediate-early; IFN, interferon; MHC, major histocompatibility complex; SFU, spot-forming unit; SOT, solid organ transplant of various types; tx, transplant.

^a Only pediatric study.

Table 3. Potential Clinical Scenarios and Frequency of Testing for Cytomegalovirus Immune Monitoring Assays

Clinical Scenario	Suggested Frequency of Immune Monitoring	Suggested Clinical Management
CMV D ⁺ /R ⁻ on primary prophylaxis	At the completion of prophylaxis and monthly for 1 y post-transplant	For negative assay, ongoing prophylaxis or frequent monitoring For positive assay, no further prophylaxis or monitoring
CMV R ⁺ with other risk factors (eg, lung transplant, ATG induction)	Preemptive strategy: monthly till 1 y posttransplant Prophylaxis: starting at completion of prophylaxis till 1 y posttransplant	For negative assay, ongoing prophylaxis or frequent monitoring For positive assay, no further prophylaxis or monitoring
Posttherapy for acute rejection	Monthly for 3 mo posttherapy	For negative assay, ongoing prophylaxis or frequent monitoring For positive assay, no further prophylaxis or monitoring
Recent completion of therapy for CMV disease (prediction of relapse)	At the completion of therapy and monthly for 3 mo	For negative assay, ongoing prophylaxis or frequent monitoring For positive assay, no further prophylaxis or monitoring
Recent completion of therapy for CMV viremia (prediction of relapse)	At the completion of therapy and monthly for 3 mo	For negative assay, ongoing prophylaxis or frequent monitoring For positive assay, no further prophylaxis or monitoring
Low-level viremia	At the onset of viremia and weekly for 3 wk	For negative assay, start therapy For positive assay continue to monitor

Abbreviation: ATG, antithymocyte globulin; CMV, cytomegalovirus.

FEASIBILITY OF TESTING IN THE CLINICAL SETTING

Despite the amount of data published in this field, there is lack of widespread clinical application. Several factors are required for immune monitoring to be feasible in the clinical setting. First, there needs to be standardization and clear cutoff values for defining positive and negative responses for immune assays. In addition, some assays require specialized equipment such as a flow cytometer or ELISpot reader. Not all hospital laboratories will have this equipment, and such assays may well need to be performed in a reference laboratory. Shipping of samples over long distances may compromise cell viability. Turnaround time for results also needs to be reasonable. Assays should have validity in various transplant types and ages. For example, the very young pediatric transplant patient with an immature immune system may have differing values for positivity. A sufficient number of lymphocytes are needed for interpretation and this may be a barrier to testing in the lymphopenic patient. Therefore, the interpretation and performance of such assays needs to be simplified as much as possible for ultimate translation to clinical practice. Finally, regulatory approval and insurance coverage of assays are also important components in bringing these assays to clinical practice.

Although immune assays may now be offered by reference laboratories, data on their interpretation are limited. The wide intra- and interindividual variability needs to be further

defined. The optimal frequency of these tests has also not been determined, although the majority of studies have performed monthly testing for variable periods in the first year posttransplant. An additional consideration is that these assays may be costly; however, cost considerations may be balanced by reduction in costs of viral load monitoring and drug cost for antiviral prophylaxis or treatment.

SUMMARY

CMV-specific CD8^{pos} T cells seem to be more important in primary infection and during the early period following transplantation, whereas CMV-specific CD4^{pos} T-cells are more important in long-term control of CMV-replication. Immune assays will need to adequately address those differences in CMV-specific T-cell response patterns. CMV-specific T cells are readily quantifiable and could be used to answer important clinical questions concerning the accurate prediction of CMV reactivation and the individual risk of developing progressive CMV replication and disease. Prophylaxis and preemptive strategies are the cornerstones of CMV prevention. However, CMV immunologic monitoring is an important advance and may add to our ability to optimally predict posttransplant CMV.

Notes

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