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A prospective longitudinal analysis of cytomegalovirus (CMV)-specific CD4+ and CD8+ T cells in kidney allograft recipients at risk of CMV infection

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Summary

Cytomegalovirus (CMV)-specific cellular immunity is essential in controlling CMV infection after transplantation. We investigated whether CMV-specific T cell levels predict CMV DNAemia after kidney transplantation. Using cytokine-flow cytometry, we enumerated interferon-γ producing CMV-specific CD4+ and CD8+ T cells at serial time points among CMV-mismatched (D+/R−) and seropositive (R+) kidney recipients who received 3 months of valganciclovir prophylaxis. Among 44 patients, eight (18%) developed CMV DNAemia at a mean (±SD) time of 151 (±33) days after transplantation, including two (5%) with CMV syndrome and three (7%) with tissue-invasive CMV disease. Cox proportional hazards regression analysis showed that CMV mismatch (D+/R−) status (HR: 13, 95% CI: 1.6–106.4; $P = 0.02$) and diabetes mellitus (HR: 5.6; 95%CI: 1.1–27.9; $P = 0.03$) were significantly associated with CMV DNAemia. In contrast, the percentage or change-over-time in CMV-specific CD4+ [pp65 ($P = 0.45$), or CMV lysate ($P = 0.22$)] and CD8+ [pp65 ($P = 0.43$), or IE-1 ($P = 0.37$)] T cells were not significantly associated with CMV DNAemia. CMV-specific T cell assays have limited clinical utility among CMV R+ kidney recipients who received valganciclovir prophylaxis. On the other hand, the clinical utility of CMV-specific T cell assays will need to be assessed in a larger cohort of CMV D+/R− kidney recipients who remain at high-risk of delayed-onset CMV disease.

Introduction

During the course of primary cytomegalovirus (CMV) infection, humans generate CMV-specific cellular immunity that controls the virus and keeps it in a latent state [1–4]. The intense pharmacologic immunosuppression after solid organ transplantation impairs the generation or reconstitution of CMV-specific cellular immunity, thereby allowing CMV reactivation and replication to go unabated leading to symptomatic clinical disease [3–6]. Among transplant recipients, CMV disease is manifested as fever and myelosuppression, which in many cases, may be accompanied by end-organ involvement such as colitis and pneumonia. In addition, CMV infection can lead to numerous indirect effects such as an increase incidence of opportunistic infections and risk of allograft failure [3–6].

The functional status of CMV-specific T cells *in vivo* correlates with clinical illness since transplant recipients

who developed asymptomatic CMV infection were characterized by robust CD4+ T cell responses, while these immune responses were delayed among patients with symptomatic disease [5]. Moreover, transplant recipients with early recovery (<30 days) of CD4+ and CD8+ T cells experienced self-resolving CMV infection, whereas those who had late recovery (>30 days) required antiviral therapy in order to clear CMV infection [7]. As a result of these clinical studies, it has been proposed that measuring CMV-specific T cells is a potentially clinically-useful strategy in assessing CMV disease predisposition after transplantation [3–6]. There are studies however that question the applicability of CMV-specific T cell measurements as predictor of subsequent risk of CMV disease in the clinical setting. A recent study evaluating CMV-specific immune reconstitution in CMV D+/R− liver recipients found no significant association between INF-γ-producing CD8+ T cells and subsequent viremia or disease [8]. These contrasting findings imply the need for more studies to define the clinical utility of CMV-specific T cell assays in the clinical setting.

The objective of this prospective study is to characterize the kinetics of CMV-specific immune reconstitution after kidney transplantation, and in the process, determine an association between absolute percentages in CMV-specific CD4+ and CD8+ T cells and the primary outcome of CMV infection and disease.

Patients and methods

Patient population

This prospective longitudinal study was conducted during a 12-month period from August 2005 to August 2006. A total of 44 adult (≥18 years) kidney recipients were enrolled. Eligible participants were CMV-seropositive (R+) or CMV-seronegative (R−) if they were to receive a kidney from CMV-seropositive donors (D+). These patients could develop primary CMV infection (among CMV D+/R− patients), reactivation infection with endogenous latent virus (among CMV R+ patients), and re-infection with *de novo* donor-transmitted virus (among CMV D+/R+ patients). All patients provided informed consent. This study was approved by the Mayo Foundation Institutional Review Board.

Clinical practice protocol

All patients received induction therapy with either antithymocyte globulin (Thymoglobulin, 1.5 mg/kg/day for three or four doses on days 0, 1, 2, and 4) or anti-interleukin 2 antibody (Basiliximab, 20 mg intravenously on days 0 and 4; or Daclizumab, 1 mg/kg intravenously on day 0). Conventionally, maintenance immunosuppression consisted of tacrolimus (to maintain levels of 10–12 ng/ml during the first month, 8–10 ng/ml during the second to the fourth month, and 6–8 ng/ml thereafter), mycophenolate mofetil (starting with 750–1000 mg twice daily, and adjusted to maintain levels of 1.5–3.5 ng/ml), and prednisone (tapering doses to reach 5 mg/day on the third month after transplantation). All patients received prophylaxis with valganciclovir (900 mg once daily, dose-adjusted based on renal function) for 90 days after transplantation.

Outcomes and clinical predictors

The primary study outcome was CMV infection, which was defined as the detection of CMV DNA in peripheral blood (termed CMV DNAemia) with the use of CMV polymerase chain reaction (PCR) assay [9,10]. CMV disease, which was defined as CMV infection (CMV DNAemia) in the presence of compatible clinical manifestations [11], was a secondary outcome. Clinical and demographic data, including age, gender, race, comorbidities, induction and maintenance immunosuppressive therapy, ABO blood group incompatibility, human leukocyte antigen (HLA) mismatch, infections and mortality were collected by reviewing medical and laboratory records.

Measurement of CMV-specific CD4+ and CD8+ T lymphocytes

Peripheral blood samples were collected into heparinized tubes from patients prior to kidney transplantation, at week 2, during months 1–3, months 4–6 and at month 12 after transplantation. The samples were stimulated with CMV antigens and CMV-specific CD4+ and CD8+ T cells were measured using cytokine (interferon-γ)-flow cytometry.

CMV antigens

Cytomegalovirus (CMV) lysate (Advanced Biotechnologies Incorporated, Columbia, MD, USA) is an inactivated form of CMV strain AD169. Pepmix CMV pp65 (JPT Peptide Technologies, Berlin, Germany) is a mixture of 138 peptides (15-mers with 11 amino acid overlaps) spanning the pp65 sequence of CMV strain AD169. Pepmix CMV immediate early-1 (IE1) peptide (JPT Peptide Technologies) contains a mixture of 120 peptides (15-mers with 11 amino acid overlaps) representing the IE-1 sequence of CMV.

Detection of interferon-γ producing CD4+ T lymphocytes

A BD Fastimmune CD4+ intracellular cytokine detection kit (BD Biosciences, San Jose, CA, USA) was used

according to manufacturer's instructions. Immediately after collection, 0.5 ml of heparinized whole blood was incubated for 2 h with CMV lysate (2 μg/500 μl of blood) or pp65 (0.5 μg/500 μl of blood) in the presence of CD28/CD49d monoclonal antibody. Unstimulated blood served as negative control while blood stimulated with *Staphylococcus aureus* enterotoxin B (SEB; 1 μg/500 m-μl of blood) was used as a positive control. Brefeldin A, which acts to halt protein transport thereby enhancing intracellular cytokine detection, was added after 2 h incubation and the blood samples were then further incubated for 4 h until EDTA (50 μl) was added to halt the stimulation process. A 100 μl aliquot of the sample was added to activated stimulated or unstimulated tubes and corresponding isotype controls, followed by a series of lysis, permeabilization and incubation with 20 μl of specific BD FastImmune anti-Hu-INF-γ FITC/CD69 PE/CD4 PerCP-Cy5.5 or corresponding isotype control. The cells were fixed in 1% parafolmaldehyde solution and the number and percentage of interferon-γ producing CD4+T cells were measured using a FACS brand flow cytometer.

Detection of interferon-γ producing CD8+T lymphocytes

The principle of and methodology for measuring interferon-γ producing CD8+ T lymphocytes is similar to that of CD4+ T cells, with few modifications. A BD Fastimmune CD8 intracellular cytokine detection kit (BD Biosciences, San Jose, CA, USA) was used. Immediately after blood collection, 0.5 ml of heparinized whole blood was incubated with IE-1 (0.5 μg/500 μl of blood) or pp65 (0.5 μg/500 μl of blood) in the presence of CD28/CD49d monoclonal antibody. Unstimulated blood and SEB (1 μg/500 m-μl of blood) served as negative and positive controls, respectively. After the addition of brefeldin A, the blood samples were incubated for 6 h. After halting the activation process with EDTA, 100-μl of blood was aliquoted to the activated stimulated or unstimulated tubes and corresponding isotype control. After a series of lysis, wash and permeabilization processes, the cell pellets were incubated for 30 min with 20 μl of specific BD FastImmune anti-Hu-INF-γ FITC/CD69 PE/CD8 PerCP-Cy5.5/CD3 APC or the corresponding isotype control. After a final wash the cell pellets were resuspended in 200 μl of 1% parafolmaldehyde in PBS. The number and percentage of interferon-γ producing CD8+T cells were measured using a FACS brand flow cytometer.

Statistical Analysis

The study population was characterized using descriptive statistics. To evaluate the association of baseline characteristics with rate of CMV DNAemia or disease, the Kaplan- Meier (KM) method and Log-Rank test were used. The percentage (%) of CMV-specific CD4+ and CD8+ T-cells stimulated with various antigens were analysed independent of each other as time-dependent variables in Cox Proportional Hazards (PH) regression. Descriptive statistics and the KM analyses were performed using SAS version 8.2 (Cary, NC) while Cox PH modeling was carried out in Splus version 8.0.1. A significance level of $\alpha = 0.05$ was used for all analyses.

Results

Patient population

The study population consisted of 44 kidney recipients with a mean age (±SD) of 53 (±12) years. The population was 50% male and mostly Caucasians [$n = 34$ (85%)] (Table 1). The donor/recipient CMV-serostatus were as follows: CMV D+/R+ [$n = 17$ (39%)], D+/R− [$n = 11$ (25%)], and D−/R+ [$n = 16$ (36%)]. The most common indication for transplantation was diabetic nephropathy with or without hypertensive nephroangiosclerosis (32%) followed by autosomal-dominant polycystic kidney disease (23%). All 44 patients received kidneys from living related ($n = 22$) or unrelated ($n = 22$) donors. One patient (2%) received kidney after pancreas transplantation. Induction immunosuppressive therapy was administered to all patients and consisted of either thymoglobulin (95%) or anti-interleukin 2 antibodies (5%). The vast majority of patients received maintenance immunosuppression with mycophenolate mofetil, tacrolimus and prednisone. Four patients developed biopsy-proven acute cellular rejection, which was treated with steroids ($n = 3$) or thymoglobulin ($n = 1$), while three had acute humoral rejection which was treated with plasma exchange, intravenous immunoglobulin, and/or splenectomy.

Incidence of CMV DNAemia and disease

During the first year after kidney transplantation, eight (18%) of the 44 patients developed the primary outcome of CMV DNAemia, including two (5%) patients who developed CMV syndrome and three (7%) who developed tissue-invasive CMV disease in the form of gastritis, colitis or nephritis. There was no patient who developed breakthrough CMV DNAemia or disease during valganciclovir prophylaxis. The mean (±SD) time-to-CMV DNAemia for the eight patients was 151 (±33) days after transplantation. CMV syndrome and CMV disease occurred at a mean [±SD (n)] of 156 [±52 ($n = 2$)] and 136 [±21 ($n = 3$)] days after transplantation, respectively. Asymptomatic CMV DNAemia was treated with oral valganciclovir (900 mg twice daily, adjusted for renal function), while CMV disease was treated with intravenous ganciclovir (5 mg/kg every 12 h, adjusted based on renal

function) or oral valganciclovir (900 mg twice daily, adjusted based on renal function). All responded to treatment with clinical and virologic resolution of CMV infection.

The KM 1 year rate of CMV DNAemia was significantly different among the three CMV D/R serogroups: 7% for CMV D+/R+ patients (one event among 17 patients at risk), 0% for CMV D−/R+ patients (zero among 16 patients at risk), and 64% for CMV D+/R− patients (seven events among 11 patients at risk) ($P < 0.001$; Log-Rank Test). Among eleven CMV D+/R− patients at risk, the KM one-year rate of CMV disease (CMV syndrome and tissue-invasive disease) was 46% (five events) compared to 0% in both the CMV D+/R+ and D−/R+ patients ($P < 0.001$; Log-Rank Test).

Kinetics of CMV-specific CD8+ and CD4+ T cells after kidney transplantation

Changes-over-time in the percentages of CMV-specific CD4+ and CD8+ T cells for all 44 kidney transplant recipients, and stratified for CMV R+ ($n = 33$) and CMV D+/R− ($n = 11$) serogroups are presented in Fig. 1. Analysed as a whole cohort (Fig. 1a and b), there was a decline from pretransplant levels in mean percentages of CMV-specific CD4+ and CD8+ T cells during the first 3 months after kidney transplantation. Since CMV D+/R− did not have pretransplant CMV-specific T cells, this pattern of decline in CMV-lysate- and pp65-activated CD4+ T cells and IE-1- and pp65-activated CD8+ T cells could be attributed wholly to CMV R+ kidney recipients (Fig 1C–F). Among CMV R+ kidney recipients, the nadir for CMV-specific CD8+ T cells activated by pp65 (mean ± SD, 0.43 ± 0.58%) or IE-1 (mean ± SD, 0.11 ± 0.18%) occurred during 1–3 months (during valganciclovir prophylaxis) and thereafter, recovered to levels higher than baseline by 6 months [for IE-1 activated cells (mean ± SD, 3.57 ± 7.82%)] or 12 months [for pp65 activated cells (mean ± SD, 3.65 ± 2.85%)] after transplantation. Likewise, the nadir for CMV-specific CD4+ T cells activated by CMV lysate (mean ± SD, 1.11 ± 1.00%) or pp65 (mean ± SD, 0.18 ± 0.23%) occurred during 1–3 months after kidney transplantation (during valganciclovir prophylaxis) among CMV R+ kidney recipients, although the recovery did not reach the baseline (pretransplant) levels by 12 months.

Expectedly, CMV D+/R− patients did not have CMV-specific T cells at baseline. Generation of CMV-specific CD4+ and CD8+ T cells in CMV D+/R− evolved gradually during the first year, and generally remained at lower levels compared to CMV R+ kidney recipients (Fig. 1C–F). Low levels of pp65- and IE-1 activated CD8+ T cells were initially detected as early as 2 weeks after transplan-

Table 1. Clinical and demographic characteristics of 44 renal transplant recipients.

Variables	*n* (%)*
Age at transplant in years, mean (±SD)	53 ± 12
Male gender	22 (50)
Caucasian	34 (85)
Indication for transplantation	
Diabetic nephropathy	10 (23)
Autosomal dominant polycystic kidney disease	10 (23)
Diabetes and hypertension	4 (9)
Hypertension	3 (7)
Glomerular disease (primary or secondary)	12 (27)
Other	5 (11)
Type of transplantation	
Kidney	43 (98)
Kidney after pancreas	1 (2)
Induction immunosuppressive therapy	
Thymoglobulin	42 (95)
Anti-interleukin 2 antibodies	2 (5)
Maintenance immunosuppressive therapy	
Mycophenolate mofetil	44 (100)
Prednisone	44 (100)
Tacrolimus	42 (95)
Sirolimus	3 (7)
Serostatus	
D+/R+	17 (39)
D−/R+	16 (36)
D+/R−	11 (25)
Positive cross-match renal transplantation	10 (23)
ABO incompatible renal transplantation	4 (9)
Number of HLA alleles mismatch	
0	3 (7)
1	2 (5)
2	9 (22)
3	8 (20)
4	4 (10)
5	11 (27)
6	4 (10)
Number of HLA-DR alleles match	
0	14 (35)
1	17 (42)
2	9 (23)
Acute rejection	
Humoral	3 (7)
Cellular†	4 (9)
CMV viremia (>1000 copies/ml)	8 (18)
CMV disease	5 (12)
Death	1 (2)

*Values are presented as number of patients (percentage), unless otherwise specified.

†One episode of acute cellular rejection preceded, while two episodes occurred after, CMV infection; one patient with acute rejection did not develop CMV infection.

ABO, blood group ABO; D, donor; HLA, human leukocyte antigen; R, recipient.

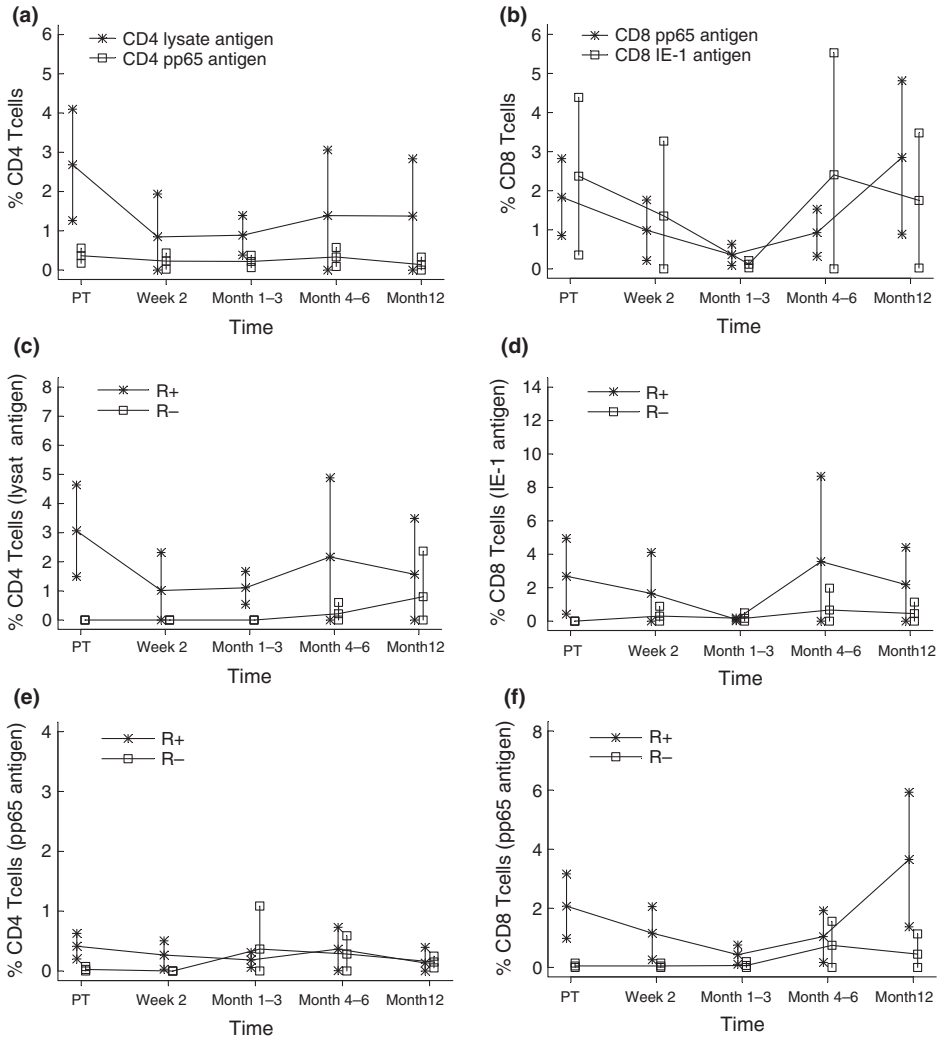

Figure 1 Percentages of CMV antigen-activated CD8+ and CD4+ T cells at different time points after kidney transplantation.

tation, and reached its highest level at 4–6 months. In contrast, CMV lysate-specific CD4+ T cells were not observed until months 4–5, and did not reach highest level until 12 months after transplantation.

Association of CMV-specific CD8+ and CD4+ T cells with CMV DNAemia

In a time-dependent analysis, neither the percentages of CD4+ T cells stimulated with CMV lysate or pp65 antigen nor those of CD8+ T cells stimulated with pp65 and IE-1 antigens were significantly associated with time-to-CMV DNAemia after kidney transplantation (Table 2). Likewise, a subgroup analysis limited to small number of CMV D+/R− kidney recipients (the group at highest risk) showed no significant association between the percentages of CD4+ T cells stimulated with CMV lysate or pp65 antigen nor those of CD8+ T cells stimulated with pp65 and IE-1 antigens with the primary outcome of time-to-CMV DNAemia or the secondary outcome of time-to-CMV disease.

Association of clinical predictors with time-to-CMV DNAemia

Clinical variables were assessed for an association with time-to-CMV DNAemia while adjusting for age (Table 3). CMV D+/R− serostatus, compared to CMV D+/R+, was significantly associated with time-to-CMV DNAemia during the first year after transplantation (HR: 13.0; 95% CI: 1.58–106.4; $P = 0.02$). Diabetes mellitus was also significantly associated with time-to-CMV DNAemia (HR 5.65; 95% CI: 1.14–27.9; $P = 0.03$). Analysis of immunosuppressive regimen as risk factors for CMV DNAemia was

Table 2. Univariate analysis of CMV-specific CD4+ and CD8+ T cells as predictors of cytomegalovirus DNAemia after kidney transplantation.

Covariate	Hazard ratio (95% CI)	*P* value
CD4+ T cells activated with CMV lysate*		
Pretransplant	0.25 (0.03–2.41)	0.23
Week 2	<0.01 (<0.01–>1000)	0.73
Months 1–3	0.06 (<0.01–22)	0.35
Months 4–6	<0.01 (<0.01–>1000)	0.60
CD4+ T cells activated with pp65 antigen*		
Pretransplant	<0.01 (<0.01–>1000)	0.53
Week 2	<0.01 (<0.01–>1000)	0.84
Months 1–3	>100 (<0.01–>1000)	0.61
Months 4–6	0.15 (<.01–81.9)	0.55
CD8+ T cells activated with pp65 antigen*		
Pretransplant	<0.01 (<0.01–12.2)	0.15
Week 2	0.03 (<0.01–41.5)	0.35
Months1–3	3.61 (0.60–21.9)	0.16
Months 4–6	<0.01 (<0.01–83.3)	0.23
CD8+ T cells activated with IE-1 antigen*		
Pretransplant	0.33 (0.02–4.34)	0.40
Week 2	<0.01 (0.00–>1000)	0.48
Months 1–3	22.8 (0.08–6377)	0.28
Months 4–6	<0.01 (0.00–>1000)	0.56
CD4+ T cells change at 2 weeks†		
CMV lysate	1.56 (0.43–5.64)	0.50
pp65 antigen	2.94 (0.09–92.6)	0.54
CD8+ T cells change at 2 weeks†		
pp65 antigen	1.07 (0.72–1.60)	0.73
IE-1 antigen	1.21 (0.58–2.55)	0.61
CD4+ T cells activated with		
CMV lysate‡	0.02 (<0.01–11.4)	0.22
pp65 antigen‡	1.54 (0.32–7.39)	0.59
CD8+ T cells activated with		
pp65 antigen‡	0.56 (0.15–2.05)	0.38
IE-1 antigen‡	0.10 (<0.01–12)	0.35

*Analysis of CD4+ and CD8+ T cell levels as single time-point measurements.
†Difference in T cell values between baseline and at 2 weeks.
‡Analysis was performed using Cox proportional hazard modeling with CD4+ and CD8+ T cells as time-dependent variables.

Table 3. Age-adjusted analysis of predictors of CMV DNAemia after kidney transplantation.

Variable	Hazard ratio (95% CIl)	*P* value
Female gender	1.19 (0.26–5.52)	0.82
Caucasian	0.87 (0.24–3.11)	0.83
Diabetes mellitus	5.65 (1.14–27.9)	0.03
Hypertension	0.24 (0.03–2.04)	0.19
Positive crossmatch	0.50 (0.06–4.09)	0.51
ABO incompatible KT	2.14 (0.26–18.0)	0.48
Plasmapheresis before or after KT	1.22 (0.26–5.72)	0.80
Plasmapheresis before vs. after KT	0.96 (0.33–2.84)	0.94
IVIG	1.22 (0.26–5.72)	0.80
HLA DR match (0, 1, 2)	0.96 (0.33–2.84)	0.94
HLA mismatch (1–6)	1.29 (0.81–2.06)	0.28
CMV serstatus*		
D+/R+	–	–
D+/R−	13.0 (1.58–106.4)	0.02

*D+/R+ serostatus is considered as a reference; IVIG, intravenous immunoglobulin; KT, kidney transplantation. Acute rejection was not assessed as a predictor of outcome since only one case preceded CMV infection.

not performed because of the almost uniform use of thymoglobulin induction and maintenance therapy with mycophenolate mofetil, tacrolimus, and prednisone.

Discussion

This prospective study, which systematically and longitudinally investigated CMV-specific CD4+ and CD8+ T cells during the first year after kidney transplantation, demonstrated clinically-relevant observations that provide insights into the kinetics of pathogen-specific T cell reconstitution and its relationship to viral reactivation. The absolute percentages of CMV-specific CD4+ and CD8+ T cells rapidly declined in CMV R+ kidney recipients to reach its nadir during 1–3 months after transplantation. The use of valganciclovir prophylaxis likely protected CMV R+ kidney transplant recipients from developing CMV disease during this at-risk period. On the other hand, generation of CMV-specific cellular immunity among CMV D+/R− patients occurs very gradually over time, and during the first year after transplantation, the levels remained lower than those of CMV R+ transplant recipients. This observation could account for the higher rate of delayed-onset CMV disease in CMV D+/R− kidney recipients despite valganciclovir prophylaxis. However, this study found no significant association between CMV-specific T cells and the time-to CMV DNAemia. Instead, CMV D+/R− serostatus was the single most important variable that was associated with delayed-onset CMV disease among kidney recipients who received valganciclovir prophylaxis.

The major focus of this study was to characterize the kinetics of CMV-specific T cell reconstitution and assess the potential clinical utility of CMV-specific CD4+ and CD8+ T cells as predictors of subsequent CMV infection after kidney transplantation. The prospective longitudinal design of this study, which required serial measurements over time, showed a decline in CMV-specific CD4+ and CD8+ T cells in CMV R+ patients to reach lowest levels during the first 3 months after transplantation. Likely the direct effect of potent immunosuppression, this decline in CMV-specific CD4+ and CD8+ T cells coincides with the traditional onset of CMV disease, which occurs during the first 3 months after transplantation in CMV R+ (and CMV D+/R−) patients who were not receiving anti-CMV

prophylaxis. Notably, no CMV R+ patient developed CMV infection during this time since they were under the protective cover of valganciclovir prophylaxis. Thereafter, no CMV R+ patient developed CMV disease and only one CMV D+/R+ patient developed asymptomatic CMV DNAemia; this implies that by the end of the 3-month prophylaxis program, CMV R+ patients have at least a partial restoration of CMV-specific cellular immunity [12,13]. The occurrence of CMV DNAemia in the CMV D+/R+ patient may also imply incomplete cross-protection against *de novo* donor-transmitted CMV strain. Indeed, CMV D+/R+ are generally at higher risk of CMV infection compared to CMV D−/R+ transplant patients [3–6]. Nonetheless, because of the low incidence of CMV infection observed in this study, CMV-specific T cell assays should not be recommended routinely among kidney recipients who received 3 months of valganciclovir prophylaxis.

In contrast, CMV D+/R− transplant recipients remained at increased risk of CMV disease after completing a 3 month valganciclovir prophylaxis program. It has been suggested that this high-risk patient population could benefit from CMV immune monitoring [14]. The ability of the high-risk CMV D+/R− patients to generate new and functional CMV-specific T cells in response to donor-transmitted CMV is likely delayed or impaired by post-transplant immunosuppression. Our study showed that CMV-specific CD4+ T cells required more than 1 year before reaching significant levels, although CMV-specific CD8+ T cell responses were detected during the first few months after kidney transplantation. Nonetheless, the quality and quantity of the initial CMV-specific CD8+ T cell responses may not have been sufficient since the majority of CMV D+/R− patients developed CMV infection or disease after discontinuing prophylaxis.

Indeed, CMV-specific T cells were not significantly associated with subsequent CMV DNAemia after kidney transplantation. While this could be due to the small number of CMV D+/R− kidney recipients in our study (and the uncommon occurrence of CMV DNAemia in CMV R+ patients), our results concur with three previous studies which found no significant correlation between CMV-specific CD4+ and CD8+ T cells and subsequent CMV infection after kidney transplantation [6]. In one study, the high CD8+ T cell responses were observed as a 'consequence' of prior CMV viremia, and thus, would not be useful 'predictor' of a later outcome [6]. Likewise, a second study showed a correlation between pp65-stimulated CD4+ and CD8+ T cells and 'concurrent', but not subsequent, CMV viremia [12]. Finally, a third study found no correlation between CMV viremia and the levels of INF-γ-producing CD8+ T cells in CMV D+/R− liver recipients [8].

Our conclusions are limited by the small number of patients who experienced CMV DNAemia. Since only one of 33 CMV R+ kidney recipients developed CMV DNAemia, the power to detect a significant association was limited. Subsequent studies should focus on the high-risk CMV D+/R− group. While our analysis of CMV D+/R− patients found no correlation between CMV T cell assays and the outcome of CMV infection and disease, this could have been due to the small number of patients. Moreover, future studies should not be restricted to interferon-γ producing T cells, since other cytokines may also be involved in CMV pathogenesis. Likewise, cost–benefit analysis and assay-standardization will be needed as these experimental measures of CMV-specific cellular immunity are translated into the clinical setting.

In conclusion, this study characterizes the kinetics of CMV-specific CD4+ and CD8+ T cells during the first year after kidney transplantation. Pharmacologic immunosuppression resulted in decline of CMV-specific CD4+ and CD8+ T cells, which would have predisposed CMV R+ patients to develop CMV reactivation; the use of valganciclovir prophylaxis during the first 3 months after transplantation may have prevented this outcome. Because CMV DNAemia is uncommon in CMV R+ patients who received valganciclovir prophylaxis, there is limited clinical application of CMV-specific T cell assays in this population. In contrast, CMV D+/R− transplant patients remain at very high risk of CMV disease despite 3 months of valganciclovir prophylaxis. While this study did not demonstrate the clinical utility of CMV-specific T cell assays in predicting their risk of CMV disease, this could be due to the small number of patients. We therefore suggest the performance of larger prospective study to address this specific issue in CMV D+/R− kidney recipients who are most vulnerable to delayed-onset CMV disease.

Authorship

AJE: designed and performed research study, collected and analysed data, and wrote the manuscript. RAB: performed research study and collected data. SKA: performed research study. BDL: analysed data and wrote part of manuscript. JEEP: analysed data and wrote part of manuscript. TSL: designed research study. RRR: designed and performed research study, collected and analysed data and wrote the manuscript.

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