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MAJOR ARTICLE

Pretransplant CMV-Specific T-Cell Immunity But Not Dose of Antithymocyte Globulin Is Associated With Recovery of Specific Immunity After Kidney Transplantation

Aurora Páez-Vega,[1,2] Sara Cantisán,[1,2,3] Maria L. Agüera,[1,4] Marta Suñer,[5] Carmen Facundo,[6] Jose R. Yuste,[2,7] Mario Fernández-Ruiz,[2,8] Miguel Montejo,[2,9] Dolores Redondo-Pachón,[10] Maria O. López-Oliva,[11] Ana Fernández-Rodríguez,[12] Maria C. Fariñas,[2,13] Domingo Hernández,[14] Oscar Len,[2,15] Patricia Muñoz,[16,17,18] Jorge Valle-Arroyo,[1,2] Cristian Rodelo-Haad,[1,4] Elisa Cordero,[2,19] and Julián Torre-Cisneros[1,2,3]

[1]Maimónides Institute for Biomedical Research of Cordoba (IMIBIC), Reina Sofía University Hospital, University of Cordoba, Cordoba, Spain, [2]Spanish Network for Research in Infectious Diseases, Instituto de Salud Carlos III, Madrid, Spain, [3]Infectious Diseases Service, Reina Sofia University Hospital, Cordoba, Spain, [4]Nephrology Service, Reina Sofia University Hospital, Cordoba, Spain, [5]Nephrology Service, Virgen del Rocío University Hospital, Seville, Spain, [6]Nephrology Service, Puigvert Fundació, Barcelona, Spain, [7]Infectious Diseases Unit, University Clinic of Navarra, Pamplona, Spain, [8]Unit of Infectious Diseases, Research Institute Hospital 12 de Octubre, Madrid, Spain, [9]Infectious Diseases Service, Cruces University Hospital, Bilbao, Spain, [10]Nephrology Service, Hospital del Mar, Barcelona, Spain, [11]Nephrology Service, La Paz University Hospital, Madrid, Spain, [12]Nephrology Service, Ramón y Cajal University Hospital, Madrid, Spain, [13]Infectious Diseases Service, Marqués de Valdecilla Hospital, University of Cantabria, Instituto de Investigación Marqués de Valdecilla, Santander, Spain, [14]Nephrology Service, Regional University Hospital "Carlos Haya," Institute for Biomedical Research of Málaga (IBIMA), Universidad de Málaga, Málaga, Spain, [15]Infectious Diseases Service, Hospital Universitari Vall d'Hebron, Barcelona, Spain, [16]Department of Clinical Microbiology and Infectious Diseases, Gregorio Marañon University Hospital; Instituto de Investigación Biomédica Gregorio Marañón; Universidad Complutense de Madrid, Madrid, Spain. CIBERES (CB06/06/0058), Madrid, Spain, [17]Instituto de Investigación Biomédica Gregorio Marañón, Madrid, Spain, [18]Department of Medicine, Universidad Complutense de Madrid, Madrid, Spain, and [19]Infectious Diseases Unit, Virgen del Rocío University Hospital, Seville, Spain

Background. This is a prospective, multicenter, observational study in cytomegalovirus (CMV)-seropositive kidney transplant recipients with pretransplant CMV-specific cell-mediated immunity (CMV-CMI) receiving antithymocyte globulin (ATG). We aimed to investigate posttransplant CMV-CMI over time and the impact of the dose-dependent ATG.

Methods. CMV-CMI was assessed at days +30, +45, +60, and +90 after transplantation with the QuantiFERON-CMV assay. A reactive result (interferon-γ [IFN-γ] ≥ 0.2 IU/mL) indicated a positive CMV-CMI.

Results. A total of 78 positive CMV-CMI patients were enrolled in the study, of which 59.5% had a positive CMV-CMI at day +30 and 82.7% at day +90. Multivariate logistic regression analysis showed that ATG dose was not associated with positive CMV-CMI at any point. However, pretransplant IFN-γ level (>12 IU/mL vs ≤12 IU/mL) was associated with positive CMV-CMI at day +30 (odds ratio, 12.9; 95% confidence interval, 3.1–53.3; $P < .001$). In addition, all the patients who did not recover CMV-CMI at day +90 had a pretransplant IFN-γ level ≤12 IU/mL.

Conclusions. More than half of CMV-seropositive kidney transplant recipients receiving ATG recover (or maintain) CMV-CMI by the first month after transplantation. The pretransplant IFN-γ level, but not the ATG dose, shows a strong association with the kinetics of this recovery.

Keywords. cytomegalovirus infection; kidney transplant; kinetics of CMV-specific cell-mediated immunity; QuantiFERON-CMV assay; antithymocyte globulin.

Antithymocyte globulin (ATG) is a potent immunosuppressive drug that acts by reducing global T-cell immunity and the incidence of acute rejection [1, 2]. Although the published evidence is contradictory [3, 4], it is generally accepted that the use of ATG is associated with a higher risk of cytomegalovirus (CMV) reactivation [5, 6]. Consequently, the clinical guidelines for managing this infection recommend universal prophylaxis regardless of the donor's CMV serology when ATG is indicated [7, 8].

It is widely accepted that antiviral prophylaxis in CMV-seropositive recipients (R^+) favors the occurrence of late-onset CMV replication and disease because it inhibits the development of CMV-specific cell-mediated immunity (CMV-CMI), which confers long-term protection from CMV reactivation [9–13]. In contrast, when a preemptive therapy strategy is used, the immune system responds to CMV replication by developing a potent positive CMV-CMI [4, 14, 15]. Additionally, the use of ATG also delays the development of CMV-CMI after transplantation, and it has been reported that this immunity can be recovered during the first 3 months after transplantation [4].

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Correspondence: Sara Cantisán, PhD, Infectious Diseases Unit, Maimónides Institute for Biomedical Research of Cordoba (IMIBIC)/Reina Sofía University Hospital/University of Cordoba, Avda, Menéndez Pidal s/n, 14004 Cordoba, Spain (sacanti@hotmail.com).

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However, the potential dose-dependent effect of ATG on CMV-CMI has not been sufficiently investigated.

The objective of this study was to investigate whether kidney transplant recipients maintain a positive CMV-CMI when they receive ATG and valganciclovir prophylaxis, as well as the impact of ATG dose on the kinetics of CMV-CMI after transplantation. This data may help us design individualized protocols for preventive management based on CMV-CMI monitoring.

METHODS

Study Population and Design

This was a prospective, multicenter, observational study. Patients from 8 centers of the Spanish Network for Research in Infectious Diseases (REIPI) and 5 centers of the Spanish Kidney Disease Network (RedInRen) were enrolled. The study was performed in accordance with the Declaration of Helsinki and an institutional review board approved it at each site. Informed consent was obtained from all participants. The aims and goals of the study were fully disclosed.

Consecutive adult (>18 years old) CMV-seropositive kidney transplant patients receiving ATG (thymoglobulin; Genzyme) and with pretransplant positive CMV-CMI were enrolled from March 2016 to October 2018. ATG was indicated following the clinical protocols of each participating center (high-risk immunological patients and donation after circulatory death). High-risk immunological patients were defined as: (1) candidates with a panel reactivity antibody (PRA) > 30%; (2) candidates with donor-specific antibodies, and (3) retransplantation with loss of allograft due to rejection. PRA was defined as the proportion of HLA antigens singly or in combination out of a panel reacting with a patient's serum. All patients received at least 1 mg/kg/day ATG induction therapy for a maximum of 10 days. Maintenance immunosuppression consisted of a combination of calcineurin inhibitors (tacrolimus or cyclosporine), mycophenolate mofetil/mycophenolic acid, and steroids.

Patients underwent CMV prophylaxis for up to 3 months after transplantation. Prophylaxis consisted of oral administration of valganciclovir (Valcyte; Roche) at standard dose (900 mg/day) adjusted according to renal function. Intravenous ganciclovir (5 mg/kg/day) was used when oral medication was not tolerated. Pretransplant CMV-CMI was assessed in samples collected either when patients were on the waiting list or the day prior to transplantation for patients receiving a graft from living donors. In addition, CMV-CMI was also determined at days +30, +45, +60, and +90 after transplantation. The CMV load follow-up after day +90 was open to the protocol of each center when clinically indicated.

Data Collection

Data were collected on demographic characteristics, retransplantation, type of dialysis, donor type, basal renal disease, PRA, immunosuppression, pretransplant donor/recipient CMV-serostatus, ATG dose and duration, late-onset CMV replication and disease, age, and sex. A senior clinical research monitor revised all data (S.C. and J.T.C.).

Determination of Anti-CMV IgG Antibodies and CMV Load

Anti-CMV IgG antibodies were analyzed at each center to determine serostatus. CMV load was analyzed in plasma or whole blood by real-time PCR using the technique implemented at each center. Peak viral load was defined as the maximum viral load in the posttransplant period.

HLA-Typing

Genomic DNA was extracted from 200 μL of blood using the commercial QIAamp DNA Blood Mini Kit (QIAgen) and automated procedure (QIAcube QIAgen). HLA typing was performed using INNO-LIPA Multiplex, which is a polymerase chain reaction- sequence-specific oligonucleotide (PCR-SSO) reverse transcription method (Fujirebio Europe). HLA alleles were determined with LIRASTM software for INNO-LIPA HLA. The sequence-specific primer (SSP) technique was used on samples that failed analysis by SSO. In these cases, HLA High Res SSP Unitray Kits were used (Invitrogen).

Determination of CMV-CMI

CMV-CMI was assessed using the QuantiFERON-CMV assay (QF), performed according to the manufacturer's instructions (Cellestis, a QIAGEN company, Melbourne, Australia). In brief, 1 mL of heparinized whole blood was collected in 3 QF blood collection tubes. The tubes contained either (1) a mix of 22 CMV peptides; (2) a negative control (no antigens); or (3) a positive mitogen control (containing phytohemagglutinin). After collection, the tubes were shaken vigorously and incubated for 16–24 hours at 37°C. Supernatants were subsequently harvested and analyzed for interferon-γ (IFN-γ) by standard enzyme-linked immunosorbent assay (ELISA). A result for the CMV antigen was reactive when the CMV antigen response minus the negative control response was equal to or higher than 0.2 IU/mL IFN-γ. Patients with a reactive result were considered as having positive CMV-CMI. According to the manufacturer's instructions, a result was indeterminate when the IFN-γ level in the CMV antigen tube minus the negative control was less than 0.2 IU/mL and the IFN-γ level in the mitogen tube (once the negative control was subtracted) was less than 0.5 IU/mL.

Statistical Analysis

The statistical analysis was performed using IBM SPSS Statistics 24.0 software (IBM Corporation, New York, NY). Categorical variables were compared using the χ^2 or Fisher tests. Quantitative variables were compared using the Mann-Whitney *U* test. A Wilcoxon test was performed to analyze changes in the median IFN-γ values between 2 consecutive time points.

Multivariate logistic regression analysis was performed to evaluate the factors associated with the presence of positive

CMV-CMI at day +30. Only relevant and confounding variables, sex, and age were selected to explain the results. Therefore ATG dose, retransplantation, and type of dialysis were included as covariables. In addition, because all the patients had positive CMV-CMI at pretransplant, we were interested in determining whether the level of pretransplant IFN-γ might have an impact on the kinetics of CMV-CMI after transplantation. Therefore, we created a new variable called, pretransplant IFN-γ. Due to a lack of linearity, the quantitative independent variables pretransplant IFN-γ and age were transformed into categorical variables using the median values as the cutoff (age, younger and older than 60; pretransplant IFN-γ, ≤12 and >12 IU/mL).

Furthermore, because all the covariables included in a multivariate analysis should be independent of each other, diagnostic tests for collinearity were performed. As collinearity between type of dialysis and retransplantation was detected, these covariables were included in 2 different multivariate analyses. Goodness-of-fit tests were performed to select the multivariate models with the best fit. Values were considered statistically significant when the *P* value was <0.05.

RESULTS

Characteristics of Patients

Table 1 shows the characteristics of the study population. A total of 78 positive CMV-CMI patients were enrolled. Two patients died at days 226 and 247 posttransplant. Six patients developed acute cellular rejection and 1 patient developed chronic humoral rejection. Sixteen (20.5%) donors were CMV-seronegative and 59 (75.6%) were CMV-seropositive. Twenty-nine patients (37.2%) were hyperimmunized (PRA > 80%).

The median total dose of ATG was 4.5 mg/kg (interquartile range [IQR], 3.2–6.6). All patients were treated with tacrolimus, mycophenolate mofetil/mycophenolic acid, and steroids. Valganciclovir was administered for a median of 90 days (83–102 days) for CMV prophylaxis.

Three patients developed self-limited episodes of asymptomatic replication during CMV prophylaxis. After prophylaxis discontinuation, clinically significant late-onset CMV replication (>1500 IU/mL in plasma or >5000 IU/mL in whole blood) occurred in 12 of the 78 patients (15.4%). Of the 12 patients with replication, 16.7% (2/12) experienced 2 episodes. The patients developed clinically significant CMV replication at a median of 143 days after transplantation (IQR, 103.5–165.7 days). The median peak viral load was 4131 IU/mL (IQR, 2477.7–7105.0 IU/mL). No patient developed CMV disease.

Longitudinal Analysis of CMV-CMI

All patients had HLA class I alleles capable of binding the CMV peptides included in the QF assay. The QF results were available for 78, 74, 69, 76, and 75 patients at pretransplant and at days +30, +45, +60 and +90, respectively. As shown in Figure 1, 44/74 (59.5%) had positive CMV-CMI at day +30, indicating that 40.5% (30/74) of the patients had lost CMV-CMI during the first month after transplantation. From this point on, the frequency of patients with positive CMV-CMI gradually increased until day +90, when 62/75 (82.7%) had positive CMV-CMI. Consequently, 17.3% of the patients had not recovered CMV-CMI at the time of prophylaxis discontinuation.

We then analyzed the kinetics of the quantitative level of IFN-γ released by $CD8^+$ T cells in the patients with positive CMV-CMI. To this end, we selected the 64 patients with consecutive sampling over all time points. As shown in Figure 2, we observed a marked decrease from pre- to posttransplantation. Specifically, the median IFN-γ value before transplantation was 10.3 IU/mL but significantly decreased to 0.3 IU/mL at day +30 (Wilcoxon test; $P < .001$). From day +45 to day +90, the median IFN-γ value increased from 0.7 to 1.3 IU/mL. Regarding the frequency of patients with an indeterminate QF assay result, which indicates a low response to CMV antigens and mitogen, the highest frequency was found at day +30 (14.8%; 11/74). This frequency gradually decreased up to day +90, when only 2.7% (2/75) had an indeterminate result.

Additionally, we analyzed the association between positive and negative CMV-CMI throughout the follow-up period and

Table 1. Characteristics of the Study Population (N = 78)

Characteristics	No. (%)
Age, y, median (IQR)	59.5 (49–65)
Sex	
Female	39 (50)
Male	39 (50)
Retransplantation	
Yes	18 (23.1)
No	60 (76.9)
Donor/recipient CMV serostatus[a]	
D^+R^+	16 (21.3)
D^-R^+	59 (78.8)
Dialysis	
Hemodialysis	59 (75.6)
Peritoneal	14 (17.9)
No dialysis	5 (6.4)
Donor type	
Living	5 (6.4)
Donor after brain death	37 (47.4)
Donor after circulatory death	36 (46.2)
Chronic kidney disease	
Unknown	16 (20.5)
Glomerulonephritis	14 (18.0)
Polycystic kidney disease (adult type)	14 (18.0)
Autoimmune	10 (12.8)
Diabetes	6 (7.7)
Hypertension	5 (6.4)
Congenital	3 (3.8)
Others	10 (12.8)
Rejection	7 (9.0)

Abbreviations: CMV, cytomegalovirus; D, donor; IQR, interquartile range; R, recipient.
[a]CMV serostatus was not available for 3 donors.

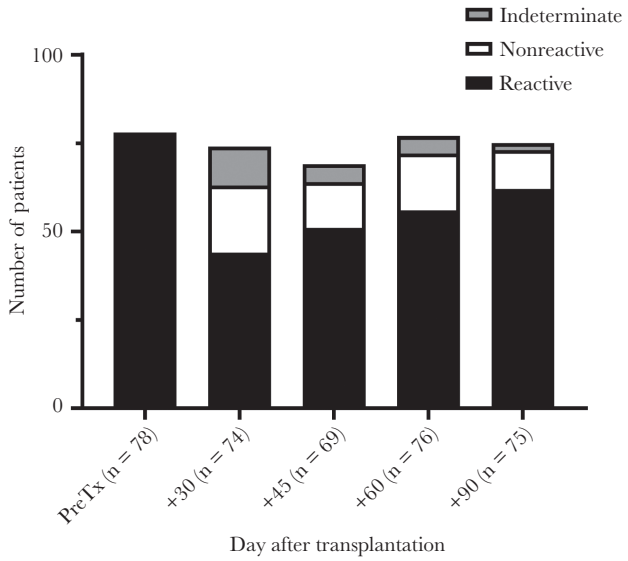

Figure 1. Number of patients with QF reactive (≥0.2 IU/mL), nonreactive, and indeterminate results at pretransplant and at days +30, +45, +60, and +90 after transplantation. Patients with QF reactive result are considered as having positive CMV-CMI. Abbreviations: CMV-CMI, cytomegalovirus-specific cell-mediated immunity; PreTx, pretransplantation; QF, QuantiFERON-CMV.

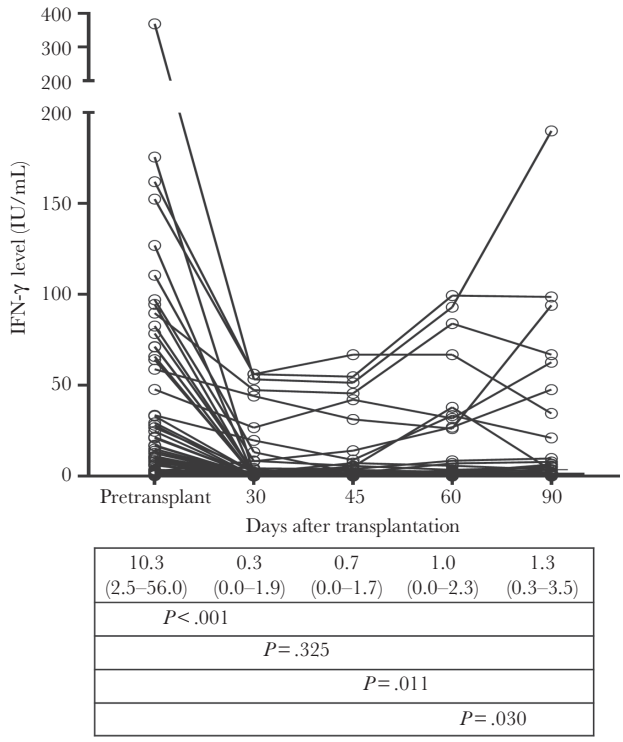

Figure 2. Individual kinetics of IFN-γ level (IU/mL) released by CMV-specific $CD8^+$ T cells measured by QF assay at pretransplant and at days +30, +45, +60, and +90 after transplantation. Only patients with consecutive sampling over all sample time points are included (n = 64). The median of IFN-γ and interquartile range at each time point are shown below the figure, as well as the *P* value of a Wilcoxon test to analyze changes in the median IFN-γ values between 2 consecutive time points. Abbreviations: CMV, cytomegalovirus; IFN-γ, interferon-γ; QF, QuantiFERON-CMV.

the incidence of clinically significant late-onset CMV replication, but no significant association was found.

Factors Associated With Early Positive CMV-CMI (Day +30)

Logistic regression was used to investigate the factors associated with early CMV-CMI recovery (at day +30) in 59.5% of the patients (Table 2; univariate analysis). Given that 2 patients (1 positive CMV-CMI and 1 negative CMV-CMI) had asymptomatic CMV replication before collecting blood samples at day +30, that CMV replication has a strong impact on CMI recovery, and that the small number of events does not permit controlling for this variable, these 2 patients were excluded from this specific analysis.

The multivariate logistic regression analysis (Table 3) included some variables such as age, sex, retransplantation, type of dialysis, pretransplant IFN-γ, ATG dose, and donor CMV-serostatus. In model 1, without the variable type of dialysis, we observed that the level of pretransplant IFN-γ showed a strong association with early positive CMV-CMI. Therefore, patients with a pretransplant IFN-γ level higher than 12 IU/mL had a 12 times higher risk of positive CMV-CMI at day +30 than patients with a level lower than or equal to 12 IU/mL (odds ratio [OR], 12.9; 95% confidence interval [CI], 3.1–53.3; $P < .001$). The total ATG dose did not show a significant association with CMV-CMI recovery at day +30 (OR, 0.8; 95% CI, .6–1.1; $P = .170$).

We then included type of dialysis in the multivariate regression analysis. However, because all the patients with a second transplantation had undergone hemodialysis and these 2 variables are therefore not independent of each other, we performed a second multivariate analysis (model 2), in which retransplantation was substituted for type of dialysis. In this new model, only pretransplant IFN-γ again showed a significant association with positive CMV-CMI at day +30 (OR, 16.8; 95% CI, 3.7–75.8; $P < .001$). The total ATG dose did not show a significant association with early CMV-CMI recovery (OR, 0.9; 95% CI, .6–1.2; $P = .547$).

Factors Associated with Late Positive CMV-CMI (Day +90)

We also analyzed whether the IFN-γ level before transplantation (lower and higher than 12 IU/mL) had any impact on the presence of positive CMV-CMI by day +90, before discontinuation of prophylaxis. Samples were not collected from 3 patients at this time. Moreover, we excluded 3 additional patients who had CMV replication before prophylaxis discontinuation. Of the 72 patients with measurable CMV-CMI at this time, 18.1% (13/72) were unable to recover CMV-CMI by day +90. All of them had a pretransplant IFN-γ level lower than 12 IU/mL.

We then performed a multivariate analysis including the same variables as in the previous analysis. However, because all the patients who were unable to recover CMV-CMI at day +90 had a pretransplant IFN-γ level ≤12 IU/mL and hence there was no variability at this point, it was not statistically possible to include the variable pretransplant IFN-γ in the

Table 2. Univariate Logistic Regression Analysis of Factors Associated With Early CMV-CMI (Day +30)[a]

Parameters	Negative CMV-CMI (n = 29)	Positive CMV-CMI (n = 43)	OR (95% CI)	*P*
Age, y, n (%)				
≤60	16 (55.2)	27 (62.8)	1	
>60	13 (44.8)	16 (37.2)	0.7 (.3–1.9)	.519
Sex, n (%)				
Female	15 (51.7)	21 (48.8)	1	
Male	14 (48.3)	22 (52.2)	1.1 (.4–2.9)	.810
Retransplantation, n (%)				
No	20 (69.0)	36 (83.7)	1	
Yes	9 (31.0)	7 (16.3)	0.4 (.1–1.3)	.145
Type of dialysis, n (%)				
Hemodialysis	20 (69.0)	34 (79.1)	1	
Peritoneal	7 (24.1)	6 (14.0)	0.5 (.1–1.7)	.272
Pretransplant IFN-γ, n (%)				
≤12 IU/mL	22 (75.9)	15 (34.9)	1	
>12 IU/mL	7 (24.1)	28 (65.1)	5.9 (2.0–16.9)	.001
ATG total dose, mg/kg, median (IQR)	4.5 (3.1–6.6)	4.0 (3.2–5.9)	0.9 (.7–1.1)	.339
Donor CMV serostatus, n (%)[b]				
D^-	5 (17.2)	9 (20.9)	1	
D^+	21 (72.4)	34 (79.1)	0.9 (.3–3.0)	.889

Abbreviations: ATG, antithymocyte globulin; CI, confidence interval; CMV-CMI, cytomegalovirus-specific cell-mediated immunity; IFN-γ, interferon-γ; IQR, interquartile range; OR, odds ratio.
[a]Two patients with low-level CMV replication before blood collection at day +30 were discarded for this analysis. QuantiFERON-CMV test result at day +30 was not available for 4 patients.
[b]CMV serostatus was not available for 3 donors.

multivariate analysis. The other variables (ATG dose, sex, age, retransplantation, and donor CMV-serostatus) were not associated with positive CMV-CMI at day +90.

Impact of ATG Total Dose and Pretransplant IFN-γ Level on Kinetics of IFN-γ Quantitative Level After Transplantation in Patients With Positive CMV-CMI

We were interested in determining whether the dose of ATG influenced the kinetics of posttransplant quantitative level of IFN-γ. To evaluate this, we selected patients with positive CMV-CMI at each time point and stratified them according to whether they had received a low or a high ATG dose using the median value of 4.5 mg/kg of ATG as the cutoff. We then compared the level of IFN-γ (IU/mL) between the low and high ATG dose groups.

As shown in Figure 3, we found significant differences between the groups only at day +30. Patients who had received a low ATG dose had a significantly higher median level of IFN-γ than patients administered a high dose (2.13 vs 0.76 IU/mL; *P* = .007). However, at day +45, +60, and +90 no significant differences were found between the groups.

Table 3. Multivariate Logistic Regression Analysis of Factors Associated With Early CMV-CMI (Day +30)[a]

Parameters	Adjusted OR	95% CI	*P*
Model 1[b]			
Age, >60 vs ≤60 y	0.5	.1–1.7	.240
Sex, male vs female	0.5	.2–1.8	.319
Retransplantation, yes vs no	0.6	.1–2.2	.428
Pretransplant IFN-γ, >12 vs ≤12 IU/mL	12.9	3.1–53.3	<.001
ATG total dose, mg/kg	0.8	.6–1.1	.170
Donor CMV-serostatus, D^+ vs D^-[c]	0.9	.2–4.0	.948
Model 2[b]			
Age, >60 vs ≤60 y	0.5	.1–2.0	.332
Sex, male vs female	0.4	.1–1.4	.153
Type of dialysis, peritoneal vs hemodialysis	0.6	.1–2.8	.501
Pretransplant IFN-γ, >12 vs ≤12 IU/mL	16.8	3.7–75.8	<.001
ATG total dose, mg/kg	0.9	.6–1.2	.547
Donor CMV serostatus, D^+ vs D^-[c]	1.2	.3–5.5	.794

Abbreviations: ATG, antithymocyte globulin; CI, confidence interval; D^+/D^-, donor cytomegalovirus seropositive/seronegative; IFN-γ, interferon-γ; OR, odds ratio.
[a]Two patients with CMV blips before blood collection at day +30 were discarded for this multivariate analysis. QuantiFERON-CMV test result at day +30 was not available for 4 patients.
[b]Goodness of fit: Hosmer-Lemeshow test result model 1, *P* = .83; model 2, *P* = .74.
[c]CMV serostatus was not available for 3 donors.

Likewise, we analyzed the impact of pretransplant IFN-γ level (≤12 IU/mL and >12 IU/mL) on the quantitative level of IFN-γ after transplantation. We found that patients with a pretransplant IFN-γ level ≤12 IU/mL showed a significantly lower level of IFN-γ throughout the follow-up period (Figure 4). The differences did not reach significance at day +30.

DISCUSSION

This prospective, multicenter study recruited CMV-seropositive kidney transplant recipients with pretransplant CMV-CMI to determine the posttransplant kinetics of CMV-CMI after receiving ATG as induction therapy and valganciclovir prophylaxis. The study shows that the level of pretransplant IFN-γ was the variable most strongly associated with early recovery (or maintenance) of positive CMV-CMI at day +30 after transplantation, whereas ATG dose was not associated with the kinetics of CMV-CMI. Additionally, no significant relationship between early CMV-CMI (at +30) and risk of late-onset CMV replication or disease was found.

Our study shows that more than 50% of CMV-seropositive recipients recover (or maintain) this immunity 1 month after transplantation. However, a high number of patients also had an indeterminate result at day +30 that was probably due to T-cell abrogation and lymphopenia driven by the ATG induction treatment. At day +90, CMV-CMI recovery was detected in an additional 25% of patients, that is 82.7% of the patients. Thus, 17.3% of transplant recipients with pretransplant positive CMV-CMI reached the time of suspending prophylaxis (day +90) without having recovered CMV-specific immunity. This percentage of patients is similar to that observed in CMV-seropositive patients not treated with ATG, thus confirming the data of other authors regarding the limited impact of ATG induction therapy on positive CMV-CMI at day +90 [4, 16, 17]. Jarque et al [17] showed that almost 50% of ATG-treated patients switched from a positive CMV-CMI to a negative CMV-CMI in the early posttransplantation period, but most achieved similar baseline CMV-CMI at 3 months posttransplantation.

The level of pretransplant IFN-γ is the variable most strongly associated with posttransplant positive CMV-CMI in our patients, which is in line with that reported by Abate et al [4]. We observed that a pretransplant IFN-γ production higher than 12 IU/mL was strongly and consistently associated with posttransplant positive CMV-CMI, and this effect remained until day +90. We cannot rule out whether this phenomenon is exclusive to patients treated with ATG or is general to any CMV-seropositive patient, because we lack an adequate control group to answer this question. ATG dose has been associated with the risk of CMV infection in kidney recipients not receiving CMV prophylaxis [18]. However, in our study, ATG dose did not show any association with positive CMV-CMI at any point.

We did not observe any significant association between donor CMV serostatus and posttransplant positive CMV-CMI in spite of the fact that donor CMV serology is known to drive the immune response in seropositive recipients [19]. However, given the limited number of CMV-seronegative donors included in

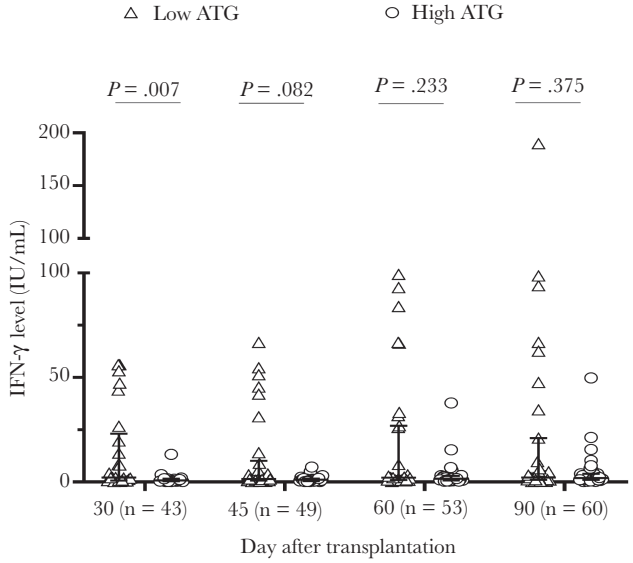

Figure 3. Impact of ATG dose on the kinetics of IFN-γ level throughout the follow-up period. Comparison of IFN-γ level released by CMV-specific $CD8^+$ T cells in patients with positive CMV-CMI receiving low and high ATG doses. The median ATG dose of 4.5 mg/kg was used as the cutoff. The median value of IFN-γ level and interquartile range are shown. The Mann-Whitney *U* test was used. Abbreviations: ATG, antithymocyte globulin; CMV-CMI, cytomegalovirus-specific cell-mediated immunity; IFN-γ, interferon-γ.

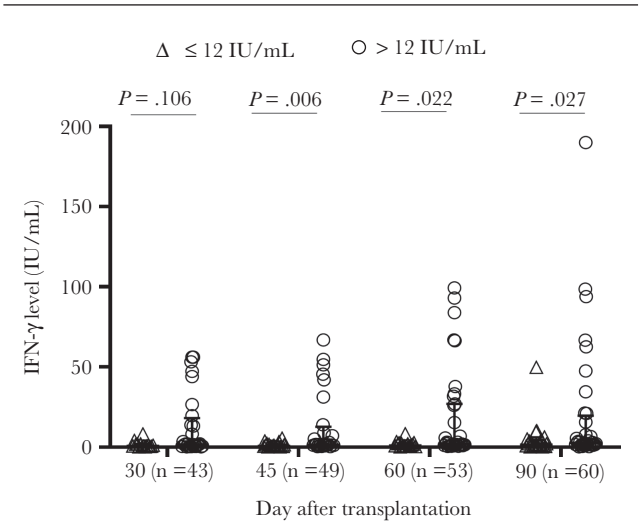

Figure 4. Impact of pretransplant IFN-γ level on the kinetics of IFN-γ level throughout the follow-up period. Comparison of IFN-γ level released by CMV-specific $CD8^+$ T cells between patients with low and high pretransplant IFN-γ level (≤12 IU/mL vs >12 IU/mL) in patients with positive CMV-CMI. The median value of IFN-γ level and interquartile range are shown. Mann-Whitney *U* test was used. Abbreviations: CMV-CMI, cytomegalovirus-specific cell-mediated immunity; IFN-γ, interferon-γ.

the study, we cannot rule out that this observation is due to an inclusion bias.

In our study, no association was observed between lack of CMV-CMI recovery and a higher incidence of late CMV replication, despite the well known association of low CMV-CMI with higher risk of CMV infection [20–24]. Jarque M et al [17] used a validation cohort to compare basiliximab and ATG-treated patients and was able to predict late-onset CMV infection according to CMV-CMI. Other authors have recently demonstrated, with a limited number of CMV-seropositive patients treated with ATG, that pp65-specific $CD4^+$ T-cell response is associated with the risk of posttransplant CMV infection [13]. In addition, in our study, no patient developed late-onset disease after prophylaxis discontinuation. This was unexpected because all the patients had received prophylaxis and it is accepted that prophylaxis inhibits the development of CMV-CMI and favors the occurrence of CMV replication and disease when suspended [4, 12, 20, 25]. In line with these reports, guidelines include the information that ATG-treated patients without CMV-CMI have a higher risk of late CMV disease [7, 8]. Given these clear results from the literature, our results should be interpreted with great caution. Our study was not designed to explore the impact of CMV-CMI on the incidence of late CMV replication or disease. Consequently, virologic monitoring after day +90, when prophylaxis was discontinued, was not in the protocol and each center followed its usual clinical practice.

Our study has some limitations. This was an observational study that lacked a control group not treated with ATG. Therefore, we do not know if many of our results will be applicable to patients not treated with ATG. Another limitation of this multicenter study is the lack of homogenization in the technique used for viral monitoring. In addition, although the QF assay is an automated technique that is easy to implement in clinical laboratory routines, it yields indeterminate results in patients with lymphopenia [26]. Therefore, it is important to validate the usefulness of this assay in clinical interventional trials.

Our results may have implications for the future design of interventional clinical trials because monitoring posttransplant CMV-CMI may allow clinicians to consider suspending valganciclovir prophylaxis when positive CMV-CMI is maintained or recovered. According to our results, this could occur in approximately 45% of patients in the first month and in an additional 15% in the second month. To apply these recommendations to clinical practice, it is essential to develop well-designed intervention studies to validate our findings. Specifically, it would be important to conduct a clinical trial to validate the strategy of premature prophylaxis suspension in patients who recover or maintain posttransplant CMV-CMI.

In conclusion, more than half of CMV-seropositive kidney recipients who receive ATG as induction therapy recover (or maintain) CMV-CMI by the first month after transplant. The basal level of IFN-γ shows a strong association with the kinetics of CMV-CMI recovery. However, we do not have enough evidence to confirm that ATG has an impact on CMV-CMI recovery in a dose-dependent manner. Our data could be useful for the design of future interventional studies to question the recommendation of universal 3-month CMV prophylaxis for all kidney transplant patients treated with ATG.

Notes

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