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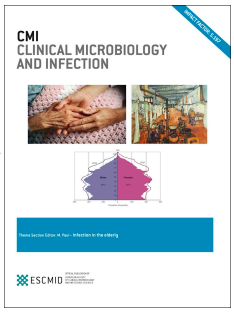

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Impact of Pretransplant CMV-Specific T-cell Immune Response in the Control of CMV Infection after Solid Organ Transplantation: a prospective cohort study

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Running title: Pretransplantation CMV-Specific Immunity.

ABSTRACT

Objectives. Although solid organ transplant (SOT) recipients with pretransplant serology for cytomegalovirus (CMV-R+) are considered at intermediate risk for CMV-infection postransplantation, CMV-infection remains a major cause of morbidity in this population. We prospectively characterized whether having pretransplant CMV-specific cellular immunity is independently associated with controlling infection after transplantation in R+ SOT recipients.

Methods. A prospective cohort of consecutive R+ SOT recipients that received pre-emptive treatment for CMV infection was monitored after transplantation and variables were recorded during the follow-up. Cytomegalovirus specific T-cell immune response was characterized by intracellular cytokine staining and viral loads determined using real-time PCR.

Results. One hundred and thirty-five R+ SOT recipients were included (67 kidney, 64 liver, 4 liver-kidney). Only one third of the patients, 42 (31.85%), had CMV specific T-cell immunity ($CD8^+CD69^+INF\text{-}\gamma^+$ T-cells >0.25%) before transplantation. Patients with negative pretransplant immunity had more CMV-infection (49, 52.7% vs. 15, 35.7% P = 0.07) and received more antiviral therapy than those with immunity (32, 34.4% vs. 6, 14.3%, P = 0.016). Having CMV specific immunity was an independent factor for protection from developing viremia ≥2,000 IU/ml (OR 0.276, 95%CI 0.105-0.725, P<0.01) and lower administration of treatment (OR 0.398, 95%CI 0.175-0.905, P=0.028). Only patients with no pretransplant CMV-specific T-cell response were diagnosed with CMV-disease (8, 8.6% vs. 0, 0%, P=0.05).

Conclusions. Our results show that having a pretransplant CMV specific T-cell response may be associated with a lower rate of CMV viremia and less antiviral treatment after transplantation; however, more prospective studies are needed to confirm these findings.

INTRODUCTION

Cytomegalovirus (CMV) infection is considered the major cause of morbidity in solid organ transplant (SOT) recipients [1]. CMV is efficiently transmitted to recipients during transplantation and the risk of infection postransplantation increases in the absence of a preexisting CMV specific immunity as well as with the amount of transplanted lymphoid tissue (being particularly high for lung and small intestine transplant) [2].

Additionally, long-term immunosuppression therapy, necessary to prevent graft rejection, poses an additional risk of CMV reactivation among recipients with previously acquired infection [2]. The pretransplant evaluation of donor and recipient serological status for CMV has been used as a marker of previous infection and as a risk factor for donor-derived transmission. It is assumed that patients with positive pretransplant CMV serology have also acquired a CMV-specific cell mediated immunity and the ability to control infection. Thus, although individual susceptibility to CMV infection is modulated by several factors (type of transplant, type of immunosuppression, or coinfection, among others), stratification according to the serological status D/R is the pillar of risk stratification. Consensus guidelines recommend that immunoglobulin G (IgG) anti-CMV antibody tests before SOT in both donors and recipients should be performed [1, 3]. The D/R serological combination differentiates between three main risk categories: i) Patients at high risk, when seronegative patients receive an organ from a seropositive donor [D+/R-]; ii) patients at intermediate risk, seropositive recipients [R+] and iii) Patients at low risk, seronegative patients that receive an organ from a seronegative donor [D-/R-] [2, 4, 5].

The immune control of CMV replication *in vivo* is primarily driven by the T-cell-mediated response, a characteristic that has been proposed as a tool to individualize and therefore to optimize antiviral treatment [6, 7], and it has been associated with spontaneous clearance of CMV viremia in patients at high (D+/R-) and intermediate risk (R+) [7-12]. Although the majority of SOT patients are R+, this is precisely the least studied population and the few published reports include an insufficient sample size [13, 14]. R+ patients have a significant incidence of CMV DNAemia (around 50%) and CMV disease (around 20%) after transplantation [15, 16]. We previously reported in Mena-Romo *et al*. that having a positive T-cell immune response at 2 weeks and 4 weeks after transplantation independently reduced the risk of requiring early treatment and developing high-level viremia. In that study, after a mid-term analysis and based on the unexpected high number of patients with no CMV-specific T-cell immune response at two weeks after transplantation, we initiated the study of the T-cell immune response before transplantation. However, the small number of patients with a pretransplant sample prevented a multivariate study to confirm if CMV-specific immunity was an independent protective factor for CMV disease [10]. Other authors reached only partial conclusions, and had little clinical utility since the CD8 T-cell response was characterized in small cohorts or with no stratification based on pretransplant serology, induction therapy or early treatment administration [19, 20].

In this context, the aim of this study was to determine whether having pretransplant CMV-specific T-cell immune response in SOT recipients with positive CMV serology (R+) is independently associated with controlling CMV infection and disease after transplantation.

PATIENTS AND METHODS

Patient follow-up. We conducted an observational prospective cohort study of consecutive SOT recipients (kidney, liver and kidney-liver) at intermediate risk for CMV infection (R+) at the University Hospital Virgen del Rocío, Seville, Spain. Patients older than 16 years were included in the study from January 2012 to May 2014 and from July 2015 to October 2017. Patients included from January 2012 to May 2014 were previously described [10]. Blood samples were collected before the transplant (the same day of transplantation), 15 days after transplantation (basal), and every two weeks during the 3 months after transplantation and monthly from the third month until one year of follow-up. A hundred and four patients were excluded, 43 (41.4%) received thymoglobulin as induction therapy, 49 (47.1%) did no sign written inform consent, 8 (7.7%) had a graft disfunction within 14 days after the transplant, 3 (2.9%) did not survive, and 1 (0.9%) had a neurological condition. The study was approved by the local Ethics Committee for Clinical Research and was conducted in accordance with the Declaration of Helsinki and the Guidelines for Good Clinical Practice.

Clinical monitoring and management. Demographics, infection-related and transplant-related variables were recorded during the follow-up period. CMV infection and disease were defined according to the GESITRA-SEIMC/REIPI recommendations for the management of CMV infection in SOT recipients based on the definitions published by Ljungman *et al*. and the International Consensus Guidelines [3, 23]. Before transplantation D/R serology for CMV was performed at the Microbiology Service using the electro-chemiluminescence immunoassay (ECLIA, Roche Products Ltd.) following the manufacturer's instructions.

All patients received an immunosuppressive regimen according to the hospital transplant protocol. In accordance with current institutional guidelines, patients received

preemptive regimens consisting of oral valganciclovir; (900 mg/12 h) or, if no oral tolerance, intravenous ganciclovir (5 mg/kg/12 h), if evidence of CMV disease or if CMV viral load was above 3,983 international units per milliliter (IU/ml) [24]. Antiviral regimens were continued until CMV viral load became undetectable in plasma. Indication for switching to m-TOR in the context of a CMV infection was recommended in the cases of persistent CMV infection that do not remit with reduction of immunosuppressors or antiviral treatment, or in cases of recurrent infection (second or third CMV-infection episode) in the same patient.

CMV viral load determination. A replication episode was considered the period with detectable viral load above the limit of detection of the assay until the first negative result. CMV viral loads were determined in plasma from January to April 2012 using the Quant CMV LightCycler® 2.0 PCR Kit (Roche Diagnostics), with a limit of detection of 180 IU/ml. From April 2012 to December 2015 the viral loads were determined using the COBAS Ampliprep/COBAS Taqman CMV test (Roche Diagnostics), with a limit of detection of 137 IU/ml, and from December 2015 to October 2017 using the COBAS 6800 (Roche Diagnostics), with a limit of detection of 34.5 IU/ml. Viral loads were standardized and expressed as IU/ml, using the WHO International Standard for Human CMV for Nucleic Acid Amplification Technique (National Institute for Biological Standards and Controls, NIBSC 09/162) [1].

CMV-specific cellular immune response determination. CMV-specific T-cell immune response was characterized using intracellular cytokine staining as previously shown [10] with no modifications. Briefly, 500 µl of freshly isolated whole blood were stimulated with 1 mg/ml of each of the peptides PepMix HCMV pp65 and PepMix HCMV IE-1 (JPT Peptides Technologies GmbH). Unstimulated samples were used as negative control, samples were stimulated with 1.5 mg/ml Streptomyces ionomycin

calcium salt, and 25 ng/ml of PMA (4-alpha-phorbol 12-myristate 13- acetate; Sigma Aldrich) were used as positive control. Samples were costimulated with 1 mg/ml CD28/CD49d (Becton Dickinson) and 10 mg/ml of brefeldin A (Becton Dickinson) was added to avoid cytokine secretion. Samples were incubated for 4 h at 37ºC with 5% CO_2 followed by an incubation for 10 min at room temperature with 5 ml of FACS Lysis Solution (Becton Dickinson). Cells were washed with PBS and processed by flow cytometry. Surface receptors were stained by incubating for 30 min in the dark with PE anti-human CD69 (0.04 mg/ml), CD4 (0.1 mg/ml), APC/Cy7 anti-human CD8 (0.1 mg/ml) and Alexa Fluor® 700 anti-human CD3 (0.5 mg/ml, Biolegend). After fixation with 50 ml of IntraPrep reagent 1 (Beckman Coulter) during 15 min, cells were washed with PBS and permeabilized with 50 ml of IntraPrep reagent 2. For intracellular cytokine staining, monoclonal antibody FITC anti-human IFN-γ (0.05 mg/ml) was used. After incubating for 15 min, cells were washed and resuspended in 300 µl of PBS. Thirty thousand events of $CD3^+$ cells from the total population of lymphocytes were analyzed on an LSR Fortessa flow cytometer. The percentages of activated $CD4^+$ and $CD8^+$ cells were normalized to the negative control. Samples were considered positive when the percentage of activated CD4+CD69+ and $CD8^+CD69^+$ T-cells expressing IFN-γ was ≥0.25% in two consecutive samples.

Statistical analysis. A descriptive analysis of the variables was performed. Categorical and continuous variables were analyzed by the Chi-square test or Fisher exact test and the Student's *t* test, Welch test or Mann-Whitney-Wilcoxon test, respectively. Association was expressed by odds ratio (OR) and the 95% confidence interval (95% CI). Due to the low frequency of CMV disease in our cohort, we used the following surrogate markers as end-points: viremia >2000 IU/ml and the initiation of antiviral treatment. Multivariate logistic regression models were used to evaluate the possible

confounding factors of the impact of CMV-specific cellular immune response in the indication of preemptive treatment and the presence of CMV viremia over 2000 IU/ml. Variables included in the model were those that showed a significant association in the bivariate analysis ($P<0.05$) such as PreTx T-cell immune response and those considered clinically relevant such as the age, the induction with Basiliximab and the rejection prior to CMV infection. In all statistical analysis, conditions of application have been checked to be applied in each test. To avoid collinearity between independent variables, only non-related variables were included in the logistic model. Variance Inflation Factor Value (VIF) was checked to control for the influence of multicollinearity. Goodness of fit using the Hosmer-Lemeshow (calibration) test and the area under the curve (AUC) (discrimination) was also checked. Results were analyzed using SPSS version 15.0 software (SPSS, Chicago, IL). Differences were considered statistically significant when *P* values were below 0.05.

RESULTS

Study population. A total of 135 R+ SOT recipients were included in the study. Three of them had graft loss (mean± SD: 20.6 ± 9.5 weeks) and 4 patients died (mean±SD: 40.5±11.1 weeks) during follow-up. The median age was 57 years (IQR 49-64), and 92 (68.1%) of the patients were male. Of the 25 patient that switched to mTOR, only 2 patients (1 kidney and 1 liver) switched to m-TOR because of persistent infection. Baseline characteristics of the patients are listed in Table 1. Of the 135 patients only one third, 42 (31.85%), had a CMV-specific immune response in the pretransplantation, while 93 (68.15%) had no pretransplant immunity (Supplementary Figure 1A). For further analysis, patients were divided in two groups based on pretransplantation CMV-specific immune response status. As shown in Table 1, no significant differences were found among baseline demographic variables analyzed between both groups of patients.

CMV-specific T-cell immune response. During the first year after the transplant the CMV-specific T-cell immune response was characterized at the indicated time points for all 135 patients included. Comparison of the evolution of the CMV-specific T-cells in both groups of patients, with (% $CD8^+CD69^+IFN\text{-}\gamma^+$ T-cell ≥0.25%) or with no (% $CD8^+CD69^+IFN\text{-}\gamma^+$ T-cell <0.25%) CMV-specific immune response before transplantation is shown in Figure 1. All 42 patients with pretransplant immunity (although during the first two weeks after the transplant there was an initial 30% reduction of patients that had no detectable CMV-specific immune response) had CMV-specific immunity after transplantation, that happened early, at a median of 3.7 weeks (IQR 2.1-4.9, Figure 1). However, in the group of 93 patients with no pretransplant immunity, a significantly lower number (46% vs. 100%, P <0.001) of patients acquired a CMV-specific immune response during the year of follow up and it occurred

significantly later than patients with pretransplant immune responses at a median of 11.4 weeks after transplantation (IQR 4.6-27, P <0.001; Figure 1).

We next analyzed and compared the kinetics of the CMV-specific $CD8^+CD69^+IFN\text{-}\gamma^+$ T-cells between patients with or with no pretransplant immunity during the first three months after transplantation. As shown in Figure 2, the percentage of $CD8^+CD69^+INF\gamma^+$ T-cells were significantly higher for all time points analyzed (basal, M1, M2 and M3) in the group of patients with pretransplant immunity compared with the group with no pretransplant immunity (P <0.001).

Incidence of CMV infection and disease. Sixty-four (47.4%) out of the 135 patients included developed at least one CMV replication episode (range 1-3) with a median peak viral load of 964.5 IU/ml (IQR 373.25-4541.5). Out of the 64 patients with viremia, preemptive treatment was indicated in 38 (59.4%) patients with a median peak viral load of 3,589.5 IU/ml (IQR 1270-7992.5). The other 26 (40.6%) patients spontaneously controlled the replication episodes with a median peak viral load of 374.5 IU/ml (IQR 298.5-674, P < 0.01) at diagnosis (Supplementary Figure 1B). A total of 28 (20.7%) patients had peak viral loads over 2000 IU/ml.

Only 8 (5.9%) transplant recipients (5 liver and 3 kidney) were diagnosed with CMV disease during the first year after transplantation: six gastroduodenal disease, one esophagitis and one hepatitis. The viral load at diagnosis ranged from 0 to 39200 IU/ml (median 1943 IU/ml (IQR 243.5-21006). Three of these episodes occurred after organ rejection and were treated with corticosteroids. All patients had a positive outcome and cleared the infection after receiving antiviral treatment.

Correlation between CMV-specific T-cell immune response and protection from CMV infection and disease. Patients with negative pretransplant CMV specific

immunity had more CMV infection (49, 52.7% *vs*. 15, 35.7% *P* = 0.07) and more frequently received antiviral therapy than those with positive immunity (32, 34.4% *vs*. 6, 14.3%, *P* = 0.016). In addition, all cases of CMV disease occurred in patients with no pretransplant CMV-specific immunity (8, 8.6% *vs*. 0, 0%, *P* = 0.05, Table 2). Six of the 8 patients that developed CMV disease never had CMV-specific immunity during follow-up. The other two patients reached $CD8^+CD69^+IFN\text{-}\gamma^+$ T-cell values> 0.25% at week 5 and 9 after transplantation, respectively.

A bivariate analysis to study the association between the timing of the episodes of replication, before or after the third month postransplantation, and having or not pretansplant cellular immune response was also performed. We found no statistical difference (Pearson Chi square p=0.701).

We performed a bivariate analysis comparing clinically or biologically relevant variables between patients with or with no high grade viremia (>2000 UI/mL) and between patient with or without indication of treatment. Having pretransplantation CMV-specific immunity was the only variable associated with developing viremia <2000 UI/mL (*P* <0.01; Supplementary Table 1) and no indication of treatment (*P*=0.01; Supplementary Table 2).

The multivariate analysis shows that having a CMV specific T-cell response before transplantation was an independent factor for protection from developing high viremia (≥2000 IU/ml, OR 0.276, 0.105-0.725, *P* <0.01) and requiring antiviral treatment (OR 0.398, 0.175-0.905, *P* = 0.028) when controlled for other possible confounding factors such as age, induction therapy and rejection prior to CMV infection (Table 3). In addition, when analysis was perfomed based on the type of organ including the interaction between organ and exposure into the model, we found no significant

differences (P=0.718 and P=0.755 for viremias >2000 IU/ml and the initiation of antiviral treatment as primary end-points, respectively).

DISCUSSION

Results of the present study show that the lack of a pretransplant CMV-specific T-cell response is an independent risk factor for requiring antiviral treatment for CMV infection in R+ SOT recipients after transplantation, since patients with no immunity did not clear infections spontaneously. This finding reveals a potential tool for defining CMV prevention strategies in patients at low risk for CMV infection and to improve their clinical management.

Pretransplant CMV serology has been used to establish patient´s risk for developing CMV infection, which guides the prophylactic approach for CMV infection in SOT recipients [3]. Several findings in the present study suggest that using CMV pretransplant serology might be insufficient. First, only one third of the SOT recipients with pretransplant positive serology for CMV also had CMV-specific T-cell immunity before transplantation. This proportion varied among different studies, ranging between 31-70% [10, 11, 21, 25-27], probably due to differences in the methods used for determining T-cell mediated immunity and the cut-offs established. In fact, the absence of standardization between centers and the complexity of the assays remain a limitation for the assessment of the CMV-specific T-cell immune response.

Second, we found that the evolution of the CMV specific T-cell response after transplantation was clearly related with having immunity before transplantation. In fact, all patients with pretransplant CMV specific T-cell responses also had immunity during the follow-up, and it was detected within the first two months after transplantation. These findings confirm our previously reported results performed in a smaller cohort of R+ patients [10]. Conversely, the evolution of the CMV-specific immune response in patients with no pretransplant immunity resembled that of CMV high-risk recipients (R-

/D+) [7, 28], since only 46% of the patients had a T-cell response after transplantation, and was detected later at a median of 3 months after the transplant.

And third, although the incidence of CMV disease was low in our cohort, only SOT recipients with no pretransplant CMV-specific T cell response developed CMV disease during follow-up.

In addition to CMV-specific T-cell immunity, other factors need to be considered in order to establish the risk of CMV infection in SOT recipients. During the past few decades, advances in the immunosuppressive regimens and treatment of acute-rejection episodes have significantly improved allograft survival after the transplant [29-31]. However, acute rejection therapy increases the risk of opportunistic infections, such as herpes virus infections [32]. Patients with therapy for acute rejection have a 13-fold higher risk of receiving CMV preemptive therapy [33]. Induction therapy with thymoglobulin has also been associated with CMV disease, and patients with thymoglobulin receive prophylaxis for CMV infection [1]. However, the use of basiliximab does not produce any change in the CMV prophylaxis strategy [1]. Although patients receiving thymoglobulin were excluded in this study, we found that induction with basiliximab increased by three-fold the risk of requiring preemptive therapy.

Some limitations of this study must be noted. First, since the pretransplant evaluation of the T-cell immune response was performed just before transplantation, we cannot establish whether samples obtained at different times before the transplant would offer similar results, since end terminal disease may affect patient's immunity, including the CMV-specific T-cell immune response. Second, the low incidence of CMV disease in our cohort probably determined that no significant association between having

pretransplant CMV-specific T-cell immune response and no developing CMV disease after transplantation was found. Although other variables expressing the absence of self-resolved CMV infection such as high viremia and preemptive therapy were used, larger studies would be needed. However, other markers, such as high viral loads, have recently been reported as a surrogate marker of CMV disease [34]. And third, we were not able to establish a cut-off higher than 0.25% that more strongly associated with protection since sensibility, specificity and negative predictive values were not high enough to be applied in clinical practice. This fact does not invalid the present study since it is the first time that it has been demonstrated that pretransplant CMV-specific cellular immunity is an independent factor associated with controlling viremias >2000IU/ml and with the administration of treatment after transplantation. These results also suggest that, although the CMV-specific immune response is important in determining the postraplantation outcome of CMV infection, it may not be sufficient and other immunological factors may be also involved.

In conclusion, our results show that having a pretransplant CMV-specific T-cell response may be associated with a lower rate of CMV viremia and less antiviral treatment after transplantation; however, clinical trials of kidney and liver R+ to evaluate the impact of the pretransplant determination of the CMV-specific T-cell immune response on the incidence of CMV disease are needed. These results may not apply to patients that received thymoglobulin as induction treatment since they were excluded from the study.

TRANSPARENCY DECLARATION

Conflict of interest: Authors declare no conflict of interest.

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Contribution: AM-O determined the CMV-specific T-cell immune response and participated in the analysis, and preparation of the manuscript. PP-R: designed and coordinated the work and the preparation of the paper. DM-R determined the CMV-specific T-cell immune response and participated in the preparation of the manuscript. CM-G participated in clinical data collection, analysis, and preparation of the manuscript. MJR, MS and CB: provided patient care and participated in the preparation of the manuscript. MS: determined the patient serology and viral loads and participated in the preparation of the manuscript. JSC participated in the analysis and the preparation of the paper. EC: provided patient care, designed and coordinated the work and the preparation of the paper.

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Table 1. Baseline characteristics of the 135 patients included with pretransplant samples.

Variable	**Total N=135**	***Patients with immunity, N=42***	***Patients without immunity, N=93***	***P***
Sex, male, n (%)	92 (68.1)	29 (69)	63 (67.7)	0.88
Age, years, mean ± SD	54.7 ± 11.86	56 ± 12.2	54 ± 11.7	0.352
Type of transplant,				
Kidney, n (%)	67 (49.6)	24 (57.1)	43 (46.2)	0.241
Liver, n (%)	64 (47.4)	17 (40.5)	47 (50.5)	0.278
Kidney-Liver, n (%)	4 (3)	1 (2.4)	3 (3.2)	0.99
Baseline immunosuppression,				
Tac-Pred-MMF, n (%)	127 (94.1)	40 (95.2)	87 (93.5)	0.99
Cicl-Pred-MMF, n (%)	8 (5.9)	2 (4.8)	6 (6.5)	0.99
Induction with Basiliximab, n (%)	53 (39.3)	21 (50)	32 (34.4)	0.086
Change to m-TOR, n (%)	25 (18.5)	6 (14.3)	19 (20.4)	0.395

Notes: SD: Standard Deviation; Tac-Pred-MMF: Tacrolimus- Prednisone- Mycophenolate Mofetil; Cicl-Pred-MMF: Ciclosporine- Prednisone- Mycophenolate Mofetil.

Table 2. Bivariate analysis comparing clinical characteristics between patients with (% $CD8^+CD69^+IFN\text{-}\gamma^+$ T-cells ≥0.25%) or without (% $CD8^+CD69^+IFN\text{-}\gamma^+$ T-cells <0.25%) pretransplant CMV-specific T-cell immune response.

Variable	Patients with immunity, N=42	Patients with no immunity, N=93	*P*
CMV infection, n (%)	15 (35.7)	49 (52.7)	0.07
Viral loads ≥2000 IU/ml, n (%)	3 (7.1)	25 (26.9)	<0.01
Nº episodes/patient, median (IQR)	0 (0-2)	1 (0-3)	0.10
Peak viral load (IU/ml), mean (SD)	1340 (5490)	3832 (14946)	0.14
Treatment, n (%)	6 (14.3)	32 (34.4)	0.016
CMV disease, n (%)	0 (0)	8 (8.6)	0.05

Notes: IU/ml: International Units per milliliter of plasma

Table 3. Multivariate analysis of possible cofounding factors associated with having CMV-specific T-cell immune response (% $CD8^+CD69^+IFN\text{-}\gamma^+$ T-cells ≥0.25) before transplantation and the development of episodes of CMV viremia ≥2000 IU/ml or the indication of treatment.

Variable	OR (CI 95%)	*P*
High viremia (≥2000 IU/ml)		
Age	1.003 (0.971-1.036)	0.844
Induction with Basiliximab	1.395 (0.639-3.043)	0.403
Rejection prior to CMV infection	3.309 (1.169-9.364)	0.024
PreTx Immunity	0.276 (0.105-0.725)	<0.01
Indication of treatment		
Age	0.994 (0.964-1.026)	0.727
Induction with Basiliximab	2.593 (1.223-5.498)	0.013
Rejection prior to CMV infection	13.664 (3.621-51.556)	<0.001
PreTx Immunity	0.398 (0.175-0.905)	0.028

Notes: OR: Odds Ratio; CI: Confidence Interval; IU/ml: International Units per mililiter of plasma; PreTX: pretransplantation

FIGURE LEGENDS

Figure 1. **CMV-specific T-cell immune response.** Evolution of the CMV-specific T-cell immune response during the first year after transplantation in patients with pretransplant CMV-specific T-cell immunity ≥0.25 (Positive PreTx immunity, continuous blue line) or patients with no pretransplant CMV-specific T-cell immunity <0.25 (Negative PreTx immunity, continuous red line). Samples analyzed were: before transplantation (PreTx), 15 days after transplantation (Basal), and monthly from 1 to 12 months (M1 through M12).

Figure 2. Kinetics of CMV-specific T-cell immune response (% of $CD8^+CD69^+IFN\text{-}\gamma^+$ T-cells). Box and whisker plot showing the kinetics of the CMV-specific T-cells at the indicated time points during the first three months after transplantation in patients with (≥0.25%; white boxes) or with no (<0.25%; grey boxes) pretransplant immunity. Levels of CD8+CD69+INFg+ at the indicated time points were compared between patients with or with no pretransplant immunity. Samples analyzed were: 15 days after transplantation (Basal), and monthly during the first 3 months (M1, M2, M3).

Figure 1

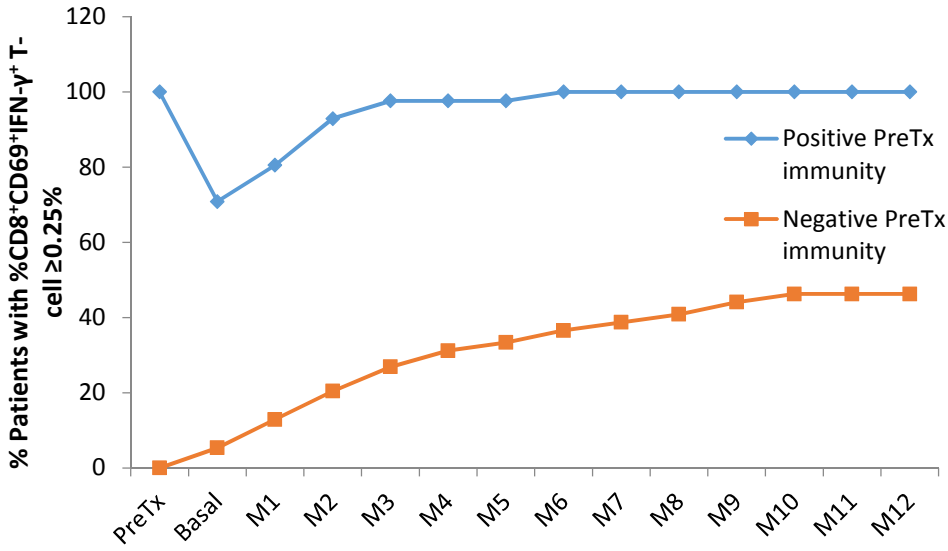

Figure 2