MAJOR ARTICLE

Evaluation of Cytomegalovirus (CMV)–Specific T Cell Immune Reconstitution Revealed That Baseline Antiviral Immunity, Prophylaxis, or Preemptive Therapy but not Antithymocyte Globulin Treatment Contribute to CMV-Specific T Cell Reconstitution in Kidney Transplant Recipients

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Background. The ultimate goal of organ transplantation is the reestablishment of organ function and the restoration of a solid immunity to prevent the assault of potentially deadly pathogens. T cell immunity is crucial in controlling cytomegalovirus (CMV) infection. It is still unknown how preexisting antiviral T cell levels, prophylaxis, or preemptive antiviral strategies and pharmacological conditioning affect immune reconstitution.

Methods. Seventy preemptively treated CMV-seropositive recipients, 13 prophylaxis-treated CMV-seronegative recipients of seropositive donor transplants, 2 seropositive recipients of seronegative donor kidneys, and 27 pretransplant subjects were enrolled in a cross-sectional study and analyzed for CMV viremia (DNAemia) and CMV-specific T cell response (interferon-γ enzyme-linked immunospot assay) before transplantation and at 30, 60, 90, 180, and 360 days after transplantation.

Results. CMV-seropositive transplant recipients displayed a progressive but heterogeneous pattern of immune reconstitution starting from day 60 after transplantation. CMV-seronegative recipients did not mount a detectable T cell response throughout the prophylaxis regimen. A single episode of CMV viremia (CMV copy number, 7000–170,000 copies/mL) was sufficient to prime a protective T cell immune response in CMV-seronegative recipients. Antithymocyte globulin treatment did not significantly affect CMV-specific T cell response.

Conclusions. Baseline immunity, antiviral therapy but not antithymocyte globulin treatments profoundly influence T cell reconstitution in kidney transplant recipients.

Kidney transplantation represents an established procedure for the treatment of end-stage renal disease. However, despite the modern advances in immunosuppression and pharmacological management, posttransplant opportunistic infections still remain a major problem in organ transplant recipients [1]. Among common opportunistic infections, cytomegalovirus (CMV) is considered to be one of the most prominent pathogens [1–5]. In kidney transplant recipients, CMV infection is associated with a variety of syndromes, and

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Table 1. General Characteristics of Patients

Characteristic	Patients (n = 112)
R^+/D^+ and R^+/D^-	70/112 (62)
R^-/D^+	13/112 (12)
R^-/D^-	2/112 (2)
Before transplantation	27/112 (24)
Sex	
Female	39/112 (35)
Male	73/112 (65)
Age, median years (range)	48 (25–70)
Immunosuppression induction	
ATG antibodies	42/85 (49)
Anti-CD25	14/85 (17)
None	29/85 (34)
Immunosuppressive maintenance	
CNI, MMF, and steroids	55/85 (65)
Including mTOR inhibitors	28/85 (33)
Other	2/85 (2)
Acute rejection episodes	
All	11/85 (13)
Grade IA R^+/D^+ and R^+/D^-	7/70 (10)
Grade IA R^-/D^+	1/13 (8)
Grade IB R^+/D^+ and R^+/D^-	3/70 (4)
Grade IB R^-/D^+	0/13 (0)
Patients who experienced posttransplant CMV DNAemia	
All	50/85 (59)
R^+	45/50 (90)
R^-	5/50 (10)
Patients with CMV disease	
All	3/85 (4)
R^+/D^+ and R^+/D^-	2/70 (3)
R^-/D^+	1/13 (7)
R^+ during preemptive treatment[a]	2/70 (3)
R^- during prophylaxis treatment[a]	1/13 (7)

NOTE. Data are proportion (%) of patients, unless otherwise indicated. ATG, antithymocyte globulin; CMV, cytomegalovirus; CNI, calcineurin inhibitors; D^-, CMV-seronegative transplant donor; D^+, CMV-seropositive transplant donor; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; R^-, CMV-seronegative transplant recipient; R^+, CMV-seropositive transplant recipient.

[a] The 2 R^+ patients who had CMV disease during preemptive treatment experienced fever, diarrhea, and nausea. The R^- patient who had CMV disease during prophylaxis experienced colitis and hepatitis.

CMV may also be involved in organ damage through CMV-mediated indirect effects. In transplant patients, CMV-attributed indirect effects include chronic allograft nephropathy, allograft rejection, and increased incidence of opportunistic infections [2, 3]. The risk and the severity of CMV infection are strictly associated with the donor (D) and recipient (R) CMV serostatus, immunosuppression regimen, and development of drug-resistant strains [1–3, 6–10]. It is well recognized that seronegative recipients (R^-) of CMV-seropositive allograft (D^+) have a higher risk of contracting symptomatic CMV infection, because in this setting CMV can replicate in the absence of adequate immune response [11]. Several studies proved that the recovery of CMV-specific $CD4^+$ and $CD8^+$ T cells is associated with long-term protection from CMV reactivation and disease and reduced impact of CMV indirect effects [12–17]. Thus, the immunologic monitoring of virus-specific T cell recovery may be helpful in determining the therapeutic strategy and identifying the group of patients who are at risk of late-onset CMV disease [8, 18–20]. In this study, we analyzed the posttransplant T cell immune reconstitution, and we present

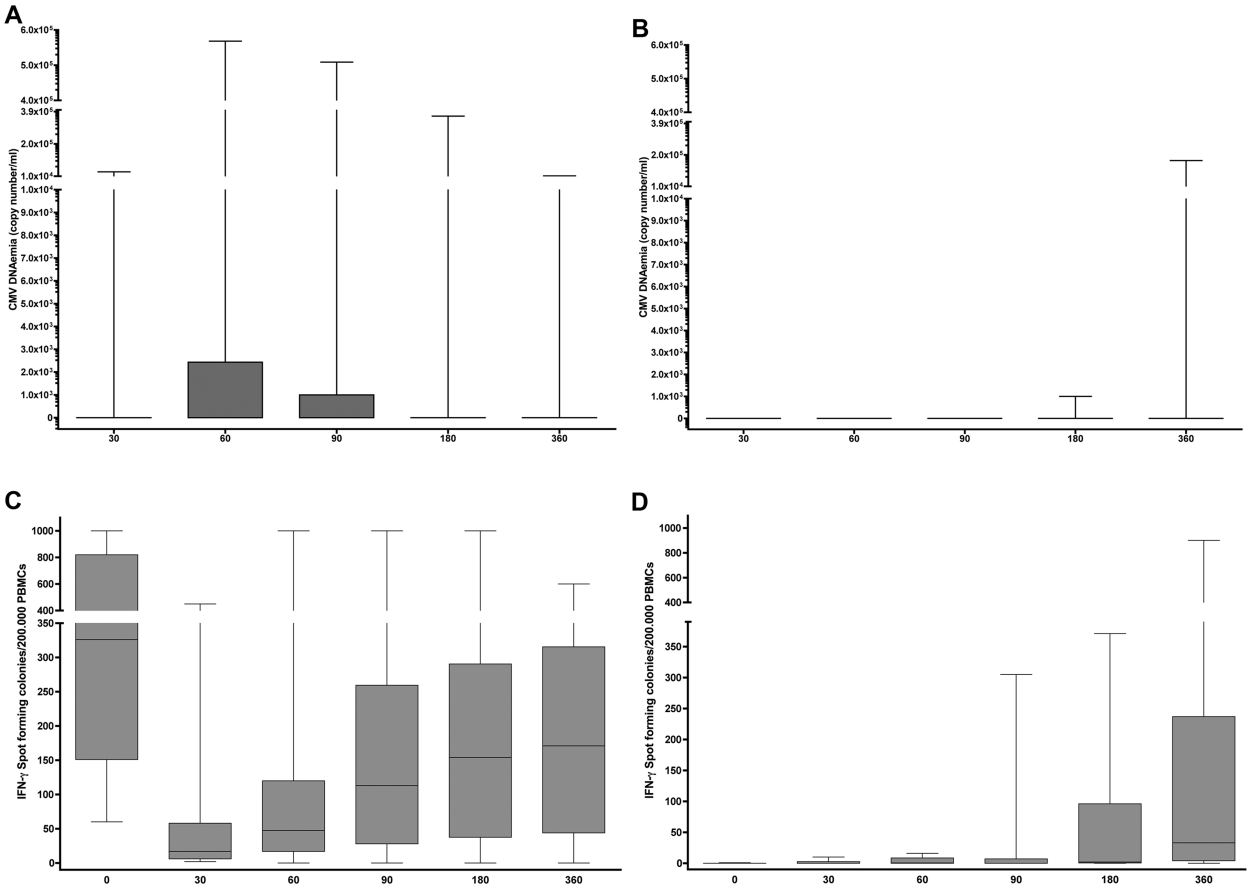

Figure 1. Cytomegalovirus (CMV) viremia and CMV-specific T cell response in kidney transplant recipients. CMV DNAemia in CMV-seropositive (*A*) and -seronegative (*B*) transplant recipients. Interferon (IFN)-γ enzyme-linked immunospot (ELISPOT) counts in CMV-seropositive (*C*) and -seronegative (*D*) transplant recipients. CMV DNAemia is expressed as copies/mL in whole blood samples. ELISPOT results are expressed as spot-forming colonies/200,000 peripheral blood mononuclear cells (PBMCs). Numbers on the *x*-axis refer to the days after transplantation.

evidence that preemptive and prophylaxis strategies and presence of baseline immunity may dramatically affect immune recovery in transplant patients. On the other hand, we show that antithymocyte globulin (ATG) treatment has limited impact on T cell reconstitution.

MATERIALS AND METHODS

Patients. Eighty-five adult kidney transplant recipients and 27 kidney pretransplant patients were enrolled in the study from September 2006 through March 2009. All 85 patients underwent kidney transplantation from September 2006 through March 2009. The cohort of 85 patients included 70 preemptively treated CMV-seropositive recipients (R^+), 13 prophylaxis-treated CMV-seronegative recipients (R^-) of seropositive donors (D^+), and 2 D^-/R^- kidney transplant recipients. Patients' demographic and clinical data are shown in Table 1. Kidney transplant recipients were voluntarily recruited among patients in the transplant list (time 0) and those at 30, 60, 90, 180, and 360 days after transplantation. Inclusion criteria for being enrolled in the study included being >18 years of age, being affected by nephropathy or being a kidney transplant recipient with no other preexisting immunodeficiency, and providing a signed Internal Review Board–approved study enrollment agreement. This study is a cross-section analysis of transplant patients. At scheduled routine medical visits corresponding with each time point, patients were voluntarily recruited and asked to donate 10 mL of peripheral blood for interferon (IFN)–γ enzyme-linked immunospot (ELISPOT) assay. Because not all patients agreed to blood withdrawal at all the scheduled time points, we obtained a mean of 3 IFN-γ ELISPOT results for each patient (maximum, 5 ELISPOT results/patient; minimum, 1 ELISPOT result/patient) within 0–360 days after transplantation. The Internal Review Board of Padua General Hospital approved all medical procedures. Patients

Table 2. Distribution and Time Line of DNAemia in Cytomegalovirus (CMV)-Seropositive and CMV-Seronegative Patients

DNAemia events after transplantation	No (%) of patients	
	CMV-seropositive patients (n = 70)	CMV-seronegative patients (n = 13)
Never experienced DNAemia	25 (36)	8 (62)
Within 100 days after transplantation	30 (43)	0 (0)
After 100 days after transplantation	8 (11)	5 (38)
Experienced CMV DNAemia both before and after 100 days	7 (10)	0 (0)

were required to provide a signed written consent to be enrolled in the study, and the aims and goals of the study were fully disclosed. A written explanation with terms and privacy policy was also provided to the enrolled patients.

When included in immunosuppression induction, treatment employing ATG (Genzyme) was administered for 4–5 days (2 mg/kg/day) and was subsequently adjusted according to white blood cell count. R^+/D^+ and R^+/D^- patients were treated according to a preemptive strategy once CMV DNAemia levels >10,000 copies/mL were detected in whole blood. R^-/D^+ patients underwent antiviral prophylaxis for up to 180 days after transplantation. D^-/R^- patients were not treated with anti-CMV drugs. CMV infection treatment included oral administration of valganciclovir (Valcyte; Roche) at the standard dose (900 mg per daily orally), corrected according to renal function. Antiviral therapy was considered to be successful when 2 consecutive negative CMV DNAemia test results were obtained. We have not detected any CMV-resistant strains among the transplant patients.

Evaluation of CMV DNAemia and CMV serology test. Routine surveillance for viral reactivation or infection included weekly determination of CMV DNAemia during the first 100 days after transplant and continued thereafter if clinically indicated. CMV DNAemia was evaluated using real time polymerase chain reaction (PCR) with an Abi Prism 7900 HT (Applied Biosystems). PCR primers and probes were described elsewhere [21]. CMV IgG and IgM serology was assessed using diagnostic grade IgG and IgM enzyme-linked immunosorbent assay (Enzygnost; Dade Behring).

Evaluation of immune response. Peripheral blood mononuclear cells (PBMCs) were extracted and purified by Ficoll (GE Healthcare). PBMCs were resuspended in RPMI-1640 medium supplemented with 10% human AB serum (Sigma Aldrich) and were seeded at a concentration of 1×10^6 cells/mL per well in an IFN-γ–coated ELISPOT plate (Autoimmun Diagnostika; AID). For each patient, duplicate wells were incubated with phytohemagglutinin (10 μg/mL; AID) or phorbol 12-myristate 13-acetate (50 ng/mL; Sigma Aldrich) and ionomycin (1 μmol/L; Sigma Aldrich), CMV pp65 peptide mix (10 μg/mL; AID), or scramble peptide mix (10 μg/mL; AID). The minimum amount of PBMCs needed to perform an ELISPOT evaluation was 1.5×10^6 cells/patient. Phytohemagglutinin and phorbol 12-myristate 13-acetate with ionomycin were considered to be positive controls, scramble peptide was considered to be a negative control, and CMV pp65 was considered to be the specific stimulus. ELISPOT images were acquired and analyzed using automated image scanner (Aelvis). IFN-γ ELISPOT results are expressed as spot forming colonies (SFCs)/200,000 PBMCs.

All results shown had background subtracted (sample minus negative control). Cytokine flow cytometry showed that pp65-specific IFN-γ–secreting cells detected using ELISPOT test corresponded to $CD4^+$ and $CD8^+$ T cells (data not shown).

Statistical analysis. Statistical analysis was performed using the Mann-Whitney nonparametric test. P values of $<.05$ were considered to be significant.

RESULTS

In this study, we performed an analysis on 85 kidney transplant recipients (70 R^+, 13 R^-, 2 D^-/R^-) and 27 pretransplant patients, evaluating both CMV DNAemia and CMV-specific T cell response at 0 (before transplantation), 30, 60, 90, 180, and 360 days after transplantation. Because we did not find relevant differences in the R^+/D^+ and R^+/D^- groups in clinical features, CMV DNAemia events, and CMV-specific T cell responses, we will refer to this group as R^+, and we will refer to R^-/D^+ patients as R^-. Two R^-/D^- patients enrolled in this study and did not experience primary CMV infection. As shown in Table 1, 11 (13%) of 85 patients experienced acute grade IA (8 patients) and IB (3 patients) rejection episodes. When recipient serostatus was considered, 7 (10%) of 70 R^+ and 1 (8%) of 13 R^- patients experienced grade IA rejection, whereas 3 (4%) of 70 R^+ and no R^- patients experienced grade IB rejection. Three patients experienced CMV disease, including 2 (3%) of 70 R^+ and 1 (7%) of 13 R^- patients. CMV disease symptoms included fever, diarrhea, and nausea for R^+ patients and colitis and hepatitis for the R^- patient. In all CMV disease cases reported, CMV DNAemia accompanied the clinical symptoms, and peak

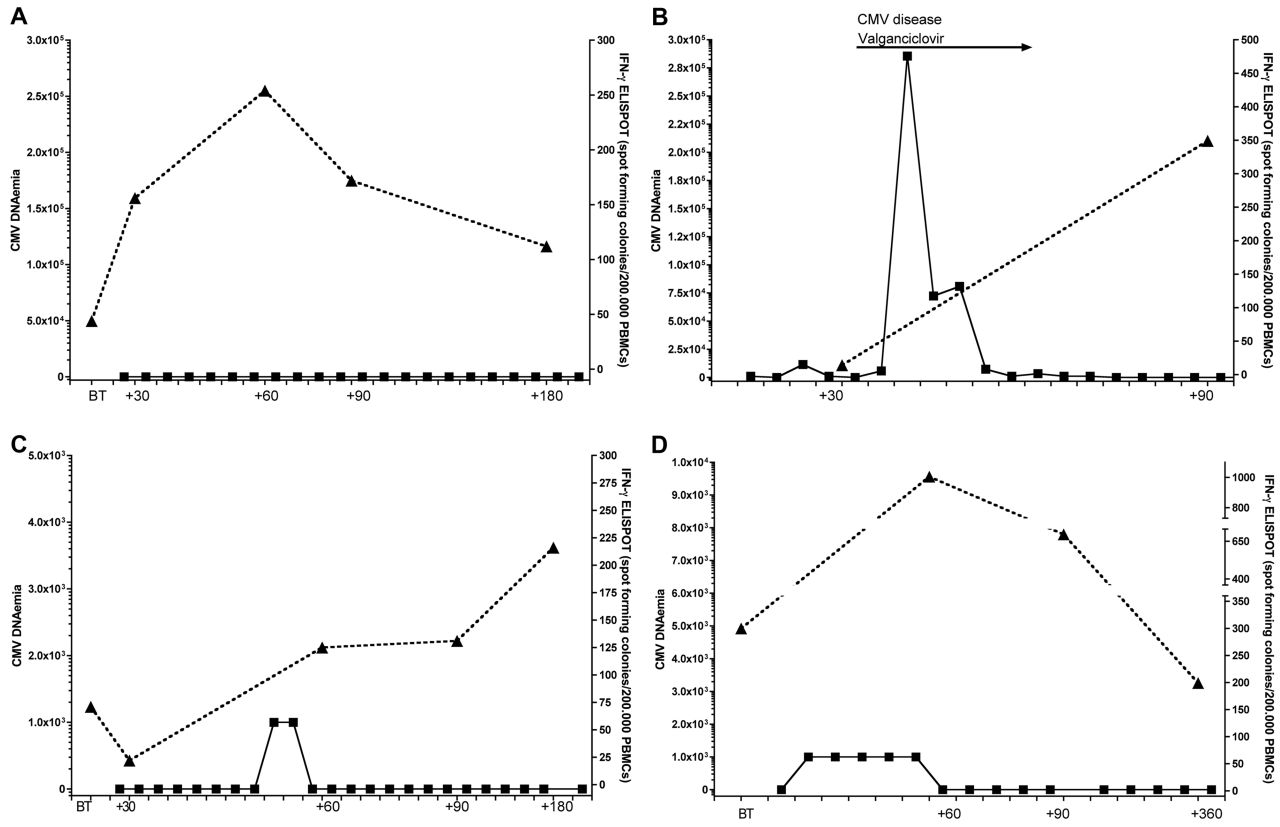

Figure 2. Pattern of immune reconstitution in 4 cytomegalovirus (CMV)-seropositive transplant recipients. Numbers on the *x*-axis refer to the days before (BT) or after transplantation. *Squares* indicate CMV DNAemia, and *triangles* indicate interferon-γ enzyme-linked immunospot assay results.

CMV DNAemia associated with CMV disease was determined to be 14,000 and 285,000 copies/mL for the R^+ patients and 166,000 copies/mL for the R^- patient. The data in Figure 1 show the CMV DNAemia episodes for R^+ patients (Figure 1*A*) and R^- patients (Figure 1*B*).

A summary of CMV DNAemia frequencies after transplantation in R^+ and R^- patients is shown in Table 2. In R^+ patients, CMV DNAemia peaked at day 60 after transplantation, with mean CMV levels exceeding 10,000 genomes/mL. Starting from day 90 onwards, we observed a steady and stable decrease in CMV DNAemia levels. As expected, R^- patients receiving antiviral prophylaxis did not show CMV DNAemia events until day 180 after transplantation, when prophylaxis was discontinued. Indeed, 5 R^- patients experienced significant episodes of CMV DNAemia when prophylaxis was discontinued. Figure 1*C* and 1*D* show CMV-specific T cell levels in R^+ and R^- patients, respectively. As expected, in R^+ patients we observed a significant decrease in CMV T cell immunity caused by immunosuppression at the time points immediately following transplantation (30 days; median IFN-γ ELISPOT, 17 SFCs/200,000 PBMCs). From day 60 (median IFN-γ ELISPOT, 48 SFCs/200,000 PBMCs) to day 360 (median IFN-γ ELISPOT, 171 SFCs/200,000 PBMCs), we observed a steady and constant CMV-specific immune reconstitution, characterized by heterogeneous values of CMV-specific T cell immunity (Figure 1*C*). Indeed, 12 and 8 R^+ patients displayed levels of antiviral immune response of 0 and 50 SFCs/200,000 PBMCs (IFN-γ ELISPOT) at days 180 and 360 after transplantation, respectively. R^- patients treated with prophylaxis presented a very different scenario of immune reconstitution; none of the patients analyzed showed evident immune reconstitution up to day 180 after transplantation. Only 5 patients gained immune reconstitution by day 360 after transplantation, and in all of them, a significant series of CMV DNAemia events anticipated the appearance of a detectable T cell immunity. Figures 2 and 3 show the kinetic of appearance of T cell response in 4 R^+ and 5 R^- patients, respectively. The reported cases are representative scenarios of immune recovery in R^+ and R^- patients. Among R^+ patients, we observed 1 case of spontaneous antiviral immune recovery with no concurrent or precedent CMV DNAemia event (Figure 2*A*). Interestingly, the patient in Figure 2*A* displayed an ELISPOT result of 151 SFCs/200,000 PBMCs at day 30 after transplantation, which is considerably higher than the median ELISPOT result of 17 SFCs/200,000 PBMCs found

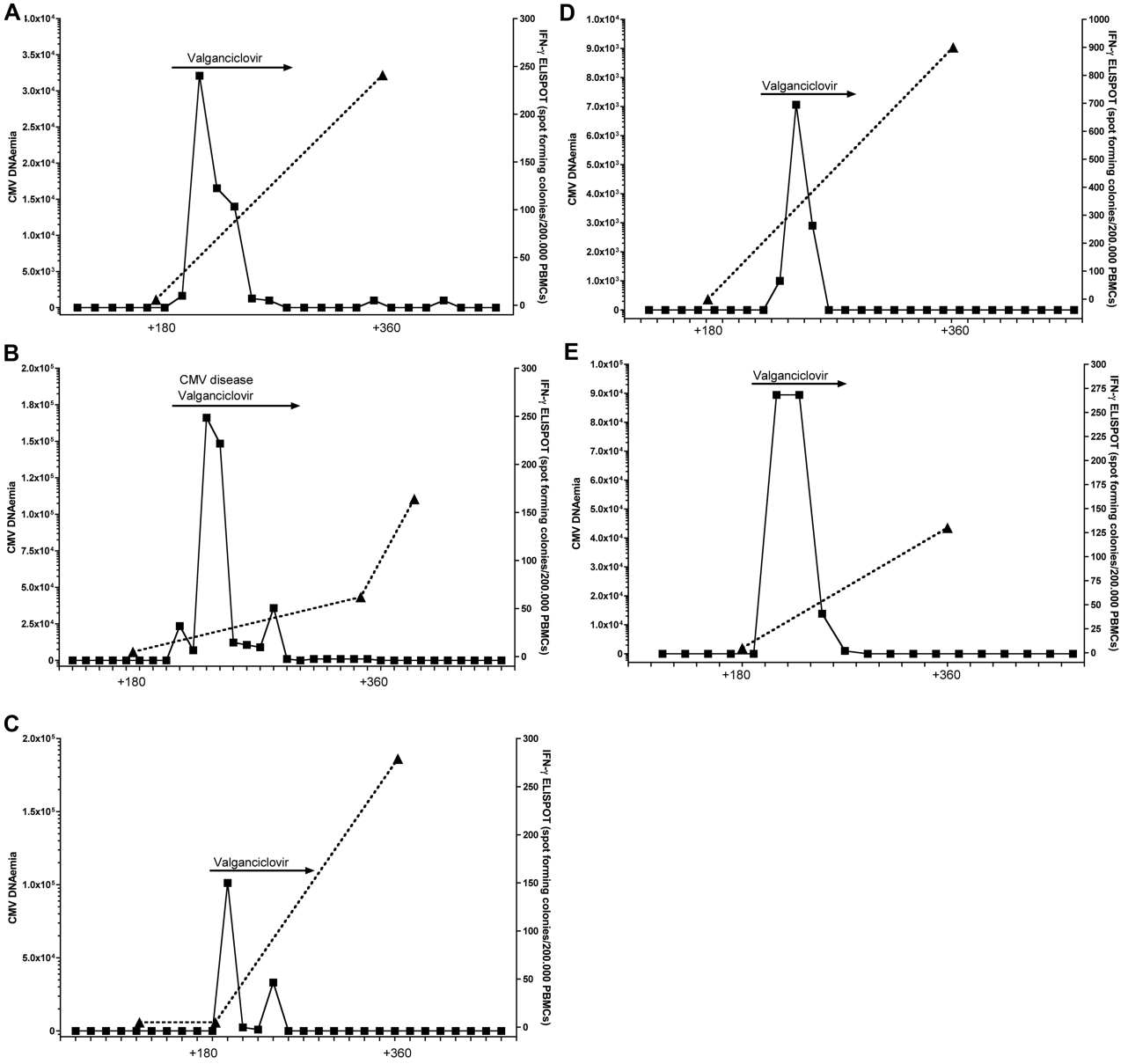

Figure 3. Pattern of immune reconstitution in 5 cytomegalovirus (CMV)-seronegative transplant recipients after CMV DNAemia. Numbers on the *x*-axis refer to the days after transplantation. *Squares* indicate CMV DNAemia, and *triangles* indicate interferon-γ enzyme-linked immunospot assay results.

at 30 days after transplantation for R^+ patients. Figure 2*B* shows 1 patient with an undetectable antiviral T cell response at day 30 after transplantation, before developing symptomatic CMV disease. This patient, following the symptomatic DNAemia episode, which was treated with valganciclovir, had an ELISPOT score of 344 SFCs/200,000 PBMCs at day 90 after transplantation. Figure 2*C* and 2*D* display 2 cases of post-CMV DNAemia immune recovery. In both cases, a low grade DNAemia, with 1000 CMV copies/mL that did not require antiviral treatment, preceded an increase in CMV-specific T cell response to ELISPOT levels of 120 and 1000 SFCs/200,000 PBMCs at day 60 after transplantation. Figure 3 shows that, in all R^- patients, a relevant CMV DNAemia event, with 7000 and 166,000 CMV copies/mL (median, 89,000 copies/mL), treated with valganciclovir was sufficient to prime detectable T cell responses on ELISPOT assay of 235 SFCs/200,000 PBMCs (Figure 3*A*), 159 (Figure 3*B*), 279 (Figure 3*C*), 896 (Figure 3*D*), and 125 (Figure 3*E*). Following the development of the T cell response, this group of patients did not experience any successive CMV DNAemia event. The patient described in Figure 3*B* developed symp-

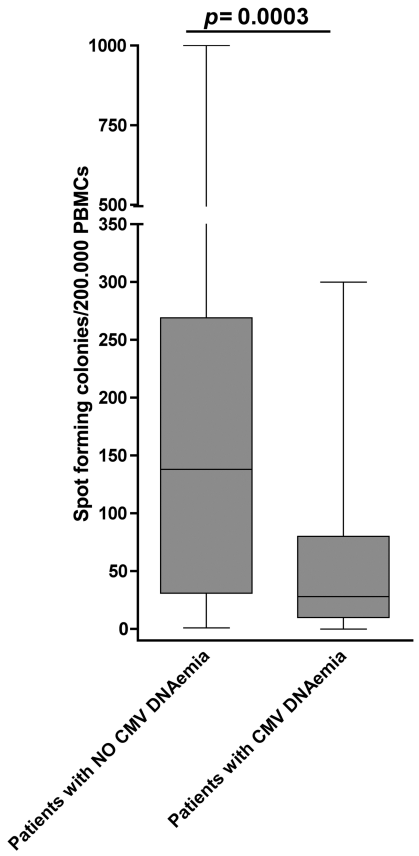

Figure 4. Relationship between enzyme-linked immunospot (ELISPOT) counts and cytomegalovirus (CMV) DNAemia in patients with or without CMV DNAemia episodes within 60 days after the ELISPOT determination. ELISPOT results are expressed as spot forming colonies/200,000 peripheral blood mononuclear cells (PBMCs).

tomatic CMV disease with fever and splenomegaly that was successfully treated with valganciclovir. None of the other described patients developed symptomatic CMV disease.

Figure 4 shows the relationship between ELISPOT results and CMV DNAemia in patients who did not experience any CMV DNAemia episode within 60 days after the ELISPOT determination versus patients who had at least 1 CMV DNAemia episode >1000 copies/mL within 60 days after the ELISPOT determination. There are statistical significant differences ($P < .001$) between the 2 groups, with patients not experiencing DNAemia displaying a median ELISPOT level of 138 SFCs/200,000 PBMCs. Patients experiencing CMV DNAemia had a median ELISPOT level of 28 SFCs/200,000 PBMCs.

To investigate the effects of ATG treatment on T cell immune reconstitution, we compared CMV-specific T cell response in preemptively treated R^+ patients treated with ATG with preemptively treated R^+ patients who did not receive ATG treatment. The data in Figure 5 show that there are not statistically significant differences in the 2 groups analyzed with regard to T cell response at any time point analyzed. We have not found significant differences in the 2 groups in terms of rejection rate, duration of antiviral treatment, or other clinically relevant data (data not shown).

DISCUSSION

The restoration of a full competent set of CMV-specific T cells is one of the most desirable achievements in solid organ transplantation, to curb the detrimental effects of CMV infection. Currently, it is still undefined how antiviral drugs may affect T cell recovery and how the therapeutic interventions may affect baseline antiviral immunity. In this study, we compared the following 2 groups of kidney transplant recipients: R^+ patients treated with preemptive therapy and R^- patients treated with prophylaxis. R^+ patients displayed a highly heterogeneous pattern in antiviral immune recovery 60–360 days after transplantation. In the general scenario of gradual and constant immune recovery found in R^+ patients, it is an interesting finding that 7 R^+ patients displayed T cell levels >100 SFCs/200,000 PBMCs by IFN-γ ELISPOT at 30 days after transplantation, when the large majority of patients had low CMV-specific T cell immunity. On the contrary, at 180–360 days after transplantation, although the large majority of R^+ patients fully recovered the antiviral T cell response, a relevant minority of R^+ patients displayed low or modest levels of CMV-specific T cell response. It is worth considering that this last group of patients might be at risk of late-onset CMV infection. These findings suggest that antiviral immune recovery in preemptively treated R^+ patients may be more rapid and efficient in some cases but slower and inefficient others. The underlying reason for this different outcome is largely unknown and may depend on genetic predisposition, susceptibility to immunosuppressive drugs, sufficient exposure to virus antigen, and other factors not yet elucidated. Given the high heterogeneity found in CMV-specific T cell levels in R^+ patients, immunological follow-up of transplant patients may be crucial for determining antiviral T cell reconstitution and, thus, for the adjustment of immunosuppressive and antiviral therapy.

Another interesting point that emerged in this study is that the median CMV-specific T cell response at day 360 (IFN-γ ELISPOT, 171 SFCs/200,000 PBMCs) is lower than that for the pretransplant group (IFN-γ ELISPOT, 326 SFCs/200,000 PBMCs) in R^+ patients. This finding suggests that the complete reestablishment of the antiviral T cell response to a pretransplant level may require >1 year after transplantation.

Prophylaxis-treated R^- patients displayed considerable differences in the immune reconstitution, compared with R^+ patients. This group of prophylaxis-treated R^- patients did not mount adequate immune reconstitution throughout the prophylaxis regimen, when virus replication was undetectable. The prophylaxis scheme has been shown to be highly effective in

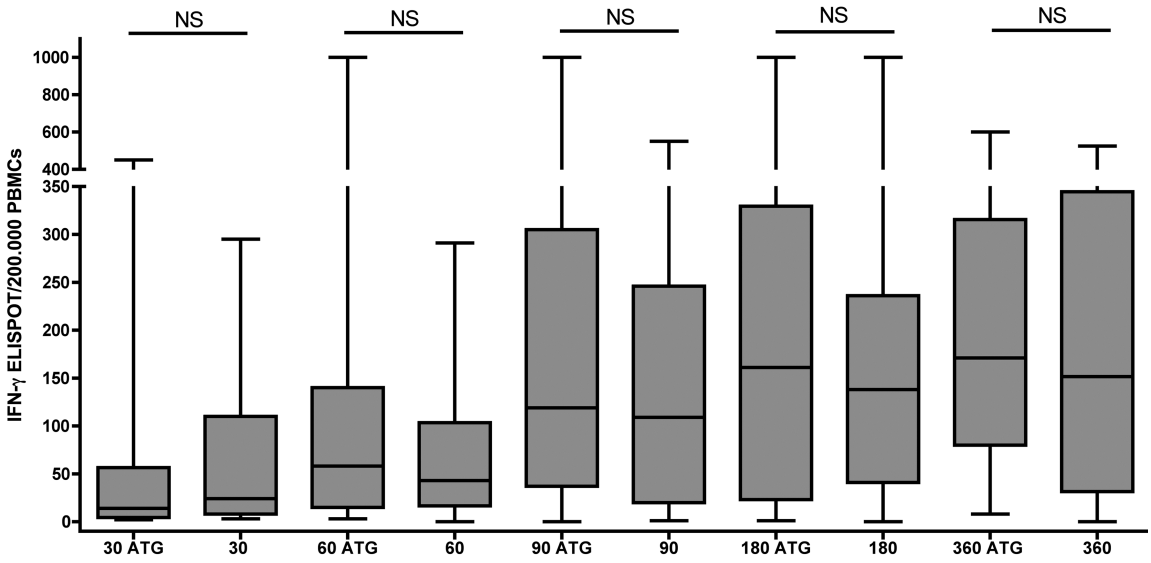

Figure 5. Effects of antithymocyte globulin (ATG) administration on immune reconstitution in cytomegalovirus-seropositive kidney transplant recipients Numbers on the *x*-axis refer to the days after transplantation. IFN-γ ELISPOT, interferon-γ enzyme-linked immunospot assay; NS, not significant; PBMC, peripheral blood mononuclear cell.

preventing CMV viremia, whereas preemptive treatment exposes patients to low-grade virus replication. Despite numerous studies and meta-analyses, the superiority of a prophylactic versus preemptive scheme is still debated [22, 23].

An interesting speculative point emerged in this study concerns virus exposure and development of CMV-specific T cell response. Is virus exposure necessary to prime a T cell response? Also, how much virus would be necessary to prime or boost a virus-specific T cell response? Is the immune recovery dependent on the restoration of preexisting immunity in R^+ patients? In this report, we presented the cases of several R^+ and R^- patients gaining T cell immunity. It appears that CMV viremia, even at low grade, may contribute to boosting T cell recovery in R^+ patients (Figure 2*B*–2*D*), and in 1 case, very low levels of T cell immunity preceded high-grade symptomatic DNAemia (Figure 2*B*). We also presented the case of 1 R^+ patient with a rapid immune recovery after transplantation who did not experience any CMV DNAemia event (Figure 2*A*). In this case, the rapid recovery of preexisting T cell levels prevented CMV viremia after transplantation.

Although in R^+ patients, virus exposure may contribute to a rapid and efficient antiviral T cell recovery, CMV viremia was required in all examined R^- patients who gained detectable CMV-specific T cell response after transplantation. Thus, the reported cases corroborate the evidence that preexisting levels of antiviral T cell immunity may be influenced by preemptive or prophylactic antiviral strategies. Exposure to virus replication, even at a low grade, may boost the antiviral immune reconstitution in preemptively treated R^+ patients, whereas the abrogation of virus replication, observed in prophylaxis-treated R^- patients, may impair virus recognition by the immune system and, thus, the efficient priming of a T cell response. However, to fully analyze and address the effects of the prophylaxis and preemptive approaches on preexisting immunity, a trial involving R^+ patients treated with antiviral prophylaxis and R^-/D^+ patients treated with a preemptive scheme may be auspicious.

At this point, it would be worthwhile to investigate the minimal and sufficient amount of virus to prime the immune response and whether this amount of virus would be safe and harmless for the patients. Recent reports suggest that CMV DNAemia levels of 300,000 genomic copies/mL may be sufficient in solid organ transplant recipients [7]; however, in our opinion this threshold is very close to the alert level where the probability of symptomatic disease increases exponentially [8]. Indeed, the patients with CMV disease reported in this study had DNAemia levels <300,000 copies/mL. It is reasonable that discrepancies attributable to conditions of DNA extraction, processing, and PCR amplification may have contributed to the precision of DNA quantification among transplantation centers [24]. This study presented evidence that DNAemia levels starting from 7000 CMV DNA copies/mL were able to prime a detectable T cell response in R^- patients when prophylaxis was discontinued. It would be intriguing and worthwhile to investigate the minimum necessary and sufficient viremia able to prime an antiviral T cell response in R^- patients.

There are several other interesting points to address. How many T cells are needed to control CMV viremia? Is there an

ELISPOT threshold predictive of viremia? We showed that there are statistically significant differences in ELISPOT counts between patients experiencing viremia versus patients who had no viremia episodes. The general scenario also suggests that high ELISPOT counts (>100 SFCs/200,000 PBMCs) prevented subsequent episodes of CMV viremia in transplant patients. But, interestingly, we have found that undetectable levels of T cell immunity were not always predictive of viremia; only 5 R^- patients (38%) with undetectable CMV ELISPOT levels developed viremia when antiviral prophylaxis was discontinued. Eight R^- patients (62%) did not experience viremia after prophylaxis interruption, despite having undetectable CMV-specific T cell levels. We speculate that in certain cases the lack of proinflammatory environment may block latent CMV reactivation. Another hypothesis is that innate immunity mechanisms may be sufficient to keep latent CMV under control. Another mechanism that may control virus replication may be the mTOR inhibitor treatments that have been previously shown to reduce the incidence of viremia [25, 26]. In this study, we have not found statistically relevant differences in CMV viremia and CMV-specific T cell reconstitution between patients treated with mTOR inhibitors and patients who received other treatments, such as mycophenolate mofetil or ATG (data not shown). It is plausible that a combination of factors, such as steroid therapy, innate immunity, and presence or absence of proinflammatory stimuli, may contribute in tilting the balance toward virus replication or virus control. To address this last question, a larger prospective study is recommended to obtain a fine and precise determination of a safe and protective T cell threshold and to assess how T cell levels control symptoms.

We also investigated the effect of ATG treatment in the cohort of R^+ patients. ATG treatment may significantly reduce renal adverse events and the toxicity of calcineurin inhibitors such as cyclosporine A; however, persisting levels of ATG caused by prolonged ATG half-life in blood may negatively affect immune recovery. In this study, we show that there are no significant differences in CMV-specific immune recovery between patients treated or not treated with ATG. ATG treatment has been shown to have multiple effects on the immune system other than lymphocyte depletion [27]. It is reasonable to speculate that ATG treatment may briefly blunt the immune system, favoring a successive recovery of immune response.

Taken together, the results show that preexisting antiviral T cell levels and prophylaxis or preemptive treatment schemes have profound effects on antiviral T cell immune reconstitution, whereas ATG treatment has a limited impact on antiviral immune reconstitution. Moreover, immunological monitoring is a reliable and vigorous tool to assess immunological reconstitution in kidney transplant recipients and may provide valid guidance for therapeutic decisions.

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