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ORIGINAL ARTICLE

Timing of CMV-specific effector memory T cells predicts viral replication and survival after allogeneic hematopoietic stem cell transplantation

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Summary

The aim of this study was to characterize timing, kinetic, and magnitude of CMV-specific immune response after hematopoietic stem cell transplantation (HSCT) and its ability to predict CMV replication and clinical outcomes. Using cell surface and intracellular cytokine staining by flow cytometry, CMV-specific T-cell response was measured in blood, while CMV viral load and chimerism were determined by real-time PCR. Patients that reconstituted CMV-specific T-cell response within 6 weeks after Allo-SCT showed a more robust immune response ($CD8^+$: 0.7 cells/μl vs. 0.3/μl; *P*-value = 0.01), less incidence of CMV replication (33% vs. 89.5%; *P*-value = 0.007), reduced viral loads (1.81 log copies/ml vs. 0 copies/ml; *P*-value = 0.04), and better overall survival (72%; CI: 0.53–0.96 vs. 42% CI: 0.24–0.71; *P*-value = 0.07) than patients with a delayed immune reconstitution. Viremic patients had significantly higher transplant-related mortality than nonviremic patients after 1 year (33% CI: 0.15–0.52 vs. 0% CI: 0.05–0.34; *P*-value = 0.01). Risk factors independently associated with viral replication were receptor pretransplant CMV-positive serostatus (*P*-value = 0.02) and acquiring CMV-specific T-cell response after 6 weeks post-transplantation (*P*-value = 0.009). In conclusion, timing of acquiring a positive CMV-specific T-cell immune response after transplantation may identify patients with different risk for viral replication and different clinical outcomes, including survival.

Introduction

Cytomegalovirus (CMV) infection is still a serious complication after allogeneic stem cell transplantation (Allo-SCT) [1]. Within the first 100 days after transplantation, using antigenemia results around 50% of the recipients develops CMV infection, while 65% to 86.5% when using real-time PCR results (RT-PCR) [2–4]. Described risk factors for CMV infection concern donor type, graft source, donor and recipient positive CMV serostatus, $CD34^+$ graft selection, conditioning regimen, incidence of acute and chronic graft-versus-host disease (GvHD), prophylaxis, and treatment of GvHD [5–9]. Preemptive antiviral therapy is administered based on detecting positive viral replication determined by either antigenemia or RT-PCR [10].

CMV-specific immune reconstitution plays a critical role in controlling viral replication [6,11,12], and it could be considered as an indicator for the functional capacity of

T cells from the graft and the recipient thymic T-cell neogenesis [13]. The absence of CMV-specific immune response has been identified as a risk factor for late CMV end-organ disease [6,9,14–16]; thus, CMV-specific $CD8^+$ T-cell levels within the first months after Allo-SCT have been proposed as a protection marker against CMV [17]. Described factors associated with a delayed immune response are prophylactic use of ganciclovir in the post-transplant period, negative CMV serostatus of the donor, use of methylprednisolone, and grades II-IV of acute GVHD [6,14]. Other studies found that steroid-induced immunosuppression and low level of $CD4^+$ and $CD8^+$ T cells inversely correlated with CMV-specific immune reconstitution and that it was enhanced after CMV infection [6,11,14,18]. However, the relationship between viral replication and CMV-specific immune reconstitution has not been completely elucidated [19].

Ljungman *et al.* in a large retrospective study showed that CMV-seropositive recipients (R+) receiving a graft from a CMV-seropositive (D+) unrelated donor had an improved 5-year survival and a reduced transplant-related mortality (TRM) compared with R+ patients who received a transplant from a seronegative donor (D−). The early immune recovery was assumed by adoptive transfer of memory T cells from the donor to the recipient in R+/D+ pairs, but unfortunately no specific cellular analysis was performed [20]. Boeckh and Nichols reviewed the impact of donor and recipient CMV serostatus on mortality and survival before SCT, suggesting that although CMV is now a rare cause of early mortality and its direct effects (such as CMV pneumonia) have been largely eradicated, eliminating the impact of CMV on survival remains elusive. Reasons include incomplete prevention of direct and indirect or immunomodulatory effects of CMV, as well as antiviral drug toxicities [21,22].

Assessing the risk related with CMV based on R/D serostatus is mostly a cohort-based strategy rather than personalized medicine. In spite of its clinical relevance, the impact of CMV-specific cellular immune response on survival has not been extensively studied in the context of prophylactic and preemptive approaches. Numerous recent studies suggest that characterization of the CMV-specific cellular immunity may be able to predict the risk of developing CMV disease [10,23]. In an era where cohorts of transplant recipients are given universal prophylaxis or preemptive therapy [7] mainly guided by the serological status, immunologic assays may allow for tailored approaches, decreasing the risk of end-organ disease, optimizing drug exposure and toxicity and likely enhancing transplant outcomes by reducing morbidity and mortality [23]. Defining the cellular immune risk for CMV infection will likely be an important cornerstone in future management strategies [24].

The goal of this study was to analyze the relationship between timing and kinetics of CMV-specific immune response with CMV replication after HSCT and to study its potential influence on clinical outcomes such as transplant-related mortality and survival.

Patients and methods

Patients

A prospective study of consecutive Allo-SCT recipients was performed between June 2008 and December 2009. Blood samples were collected (one EDTA to monitor CMV viral load and one Na-heparin to determine CMV-specific immune response and hematopoietic chimerism) at 1 week before transplant, every week during the first 3 months, every other week between 3 and 6 months, and monthly from month seven to 1-year follow-up after transplantation. The study was approved by the local Ethics Committee for Clinical Research, and all included patients signed written informed consent.

Clinical variables and management

Pretransplant demographics and graft-related variables, post-transplant clinical parameters, and complications were prospectively recorded. Patients were preemptively treated with oral valganciclovir (900 mg/12 h) or intravenous ganciclovir (5 mg/kg/12 h) when no oral tolerance, if CMV load was over 1000 copies/ml or evidence of CMV disease symptoms. Doses were adjusted in patients with renal failure. Treatment was maintained for at least 1 week after viral load reached undetectable levels. CMV infection and disease were defined as described by Ljungman *et al.* [7]. All patients received CMV-safe blood products. Graft-versus-host disease was diagnosed according to published criteria [25,26].

CMV serology, viral load, and specific immune response determinations

Serological testing for anti-CMV IgG and IgM was performed using the electrochemiluminiscence immunoassay (ECLIA, Roche Products Ltd.) following the manufacturer's instructions.

CMV viral loads were determined by RT-PCR using the Affigene® DNA Extraction kit and CMV trender assay (Cepheid AB, Bromma, Sweden) as previously shown [27,28].

CMV-specific T-cell response was determined by the identification of specific $CD4^+$ and $CD8^+$ T cells through cell surface molecule (CD4, CD8, CD3, CD69) and intracellular cytokine (IFN-γ, IL-2, IL-4) staining using flow cytometry. The frequency of CMV-specific T cells in response to CMV-pepmix stimulation was measured as previously described [27]. The percentage of $CD4^+$, $CD8^+$, and $CD3^+$ T cells that secreted IFN- γ, IL-2 and expressed

CD69 were normalized to the negative controls. Samples were considered positive when $CD4^+$ and $CD8^+$ T cells expressing CD69 was over 1% and $CD8^+$ T cells secreting IFN-γ was over 0.25%, normalized to the total number of $CD3^+$ T cells as previously described [29].

$CD8^+$ T-cell chimerism analysis

Before Allo-SCT, donor and recipient peripheral blood samples were used to extract genomic DNA. At the time of a positive CMV-specific immune response, 3.5 ml of peripheral blood was used for the positive selection of the $CD8^+$ T-cell population using the Human Whole Blood $CD8^+$ Positive Selection Kit (EasySep, StemCell Technologies, USA) following the manufacturer's instructions. Genomic DNA was extracted, from both peripheral blood and positive-selected $CD8^+$ T cells, using QIAmp DNA Mini Kit (Qiagen, Hilden, Germany). Genotypes were performed on a LightCycler 2.0 (Roche Diagnostic, Mannheim, Germany) as previously described [30].

Statistical analysis

Data with non-normal distribution were expressed as median values (range). Chi-square or Fisher's exact tests were used to compare differences between groups of categorical data. CD69 expression and cytokine secretion were compared in both $CD4^+$ and $CD8^+$ T cells between the different time points by Wilcoxon test. CMV viral load reduction without treatment administration was compared by Wilcoxon test. Risk factor analysis was performed using a logistic-regression test. Logistic-regression analyses were performed to control the effects of the analyzed clinical variables on delayed CMV-specific immune reconstitution and viral replication. A predefined set of variables was included in the multivariate analysis performed to identify those factors with a significant impact on delayed CMV-specific immune reconstitution and viral replication. The results of univariate and multivariate logistic-regression analyses were reported as odds ratios with 95 percent confidence intervals. Correlation between the acquisition of CMV-specific immune response and the decrease of the incidence of CMV viral load were compared by Pearson test. Differences in survival were compared by a log-rank test. Differences were considered statistically significant for P-values < 0.05. All statistical analyses were performed using SPSS 16.0 software (Chicago, IL).

Table 1. Clinical and biological characteristics of the study population.

Characteristic	
Patients, *n*	46
Age, mean years (range)	34 (15–61)
Sex, *n* (%)	
Male	25 (54.3%)
Female	21 (45.7%)
Underlying disease, *n* (%)	
Acute Myeloid Leukemia	20 (43.5%)
Acute Lymphoblastic Leukemia	6 (13%)
Severe Aplastic Anemia	6 (13%)
Hodgkin Disease	4 (8.5%
No-Hodgkin Lymphoma	3 (6.5%)
Chronic Myeloid Leukemia	3 (6.5%)
Myelodysplastic Syndrome	2 (4.5%)
Chronic Lymphocytic leukemia	2 (4.5%)
Disease stage at transplant, *n* (%)	
Early	20 (43.5%)
Advance	26 (56.5%)
Stem cell source, *n* (%)	
Bone marrow	4 (8.7)
Peripheral blood	34 (73.9)
Cord blood	8 (17.4)
Donor type, *n* (%)	
Matched Sibling	22 (47.8)
Unrelated	22 (47.8)
Haploidentical relative	2 (4.3)
Donor/recipient CMV serostatus, *n*	
D+/R+	23
D−/R+	14
D+/R−	4
D−/R−	5
Conditioning regimen, *n* (%)	
Myeloablative	26 (56.5)
Non myeloablative	20 (43.5)
GvHD prophylaxis, *n* (%)	
CSA + MTX	30 (65.2)
CSA + MMF	13 (28.3)
Others	3 (6.5)
Acute GvHD grade, *n* (%)	
0 to I	19 (41.3)
II to IV	27 (58.7)
Chronic GVHD, *n* (%)	
No or Limited	29 (63%)
Extensive	17 (37%)
Steroids therapy for acute GvHD grade II–IV or Extensive Chronic GvHD, *n* (%)	
Yes	37 (80.4)
No	9 (19.6)

CMV, cytomegalovirus; D, donor; R, recipient; CSA, cyclosporine; MTX, methotrexate; MMF, mofetil mycophenolate; GvHD, graft-versus-host disease.

Results

Patient characteristics

A total of 46 patients were included in the study (Table 1) with a median age of 34 years (range: 15–61). Diagnosis were acute myeloblastic leukemia ($n = 20$; 43.5%), acute lymphoblastic leukemia ($n = 6$; 13%), severe aplastic anemia ($n = 6$; 13%), Hodgkin disease ($n = 4$; 8.5%), non-Hodgkin lymphoma ($n = 3$; 6.5%), chronic myeloid

leukemia (n = 3; 6.5%), myelodysplastic syndrome (n = 2; 4.5%), and chronic lymphocytic leukemia (n = 2; 4.5%). Conditioning regimens were myeloablative in 56.5% of the cases and of reduced-intensity in 43.5%. Graft-versus-host disease prophylaxis consisted of cyclosporine (CsA) plus methotrexate (MTX) (65.2%), CsA plus mofetil mycophenolate (MMF) (28.3%), or others (6.5%). The cumulative incidence of acute GvHD of grade II-IV was 58.7% (10%, grades III–IV) and of chronic extensive GvHD was 37%. Primary treatment of GvHD was steroids plus CsA or tacrolimus and/or MMF. Five pairs of both donors and recipients negative for CMV serostatus were excluded of the following analyses when appropriate.

CMV-specific immune reconstitution and CMV replication

Overall, 24 patients developed viremia after the transplant (viremic patients). CMV replication episodes increased rapidly after the first week of transplantation, and all 24 patients had experienced CMV replication by week 9 (Fig. 1). After week 12, the incidence of replication decreased progressively until week 50, after which, no patients experienced new replication episodes.

Eight patients developed CMV-specific T-cell immune response at week 2 after transplantation, and all patients had positive immune response by week 20 (median of 5.5 weeks; range: 3–8; Fig. 1). Decline in the incidence of CMV replication inversely correlated with acquisition of CMV-specific T-cell response (Linear regression r^2 = 0.925, Pearson correlation = −0.963; P-value = 0.01; Fig. 1). Additionally, viral loads of episodes after acquisition of CMV-specific immune response were significantly lower (1.81 log copies/ml vs. 0 copies/ml; P-value = 0.04). Median time between acquisition of CMV-specific immune response and complete viral suppression was 9 weeks (range: 0–46).

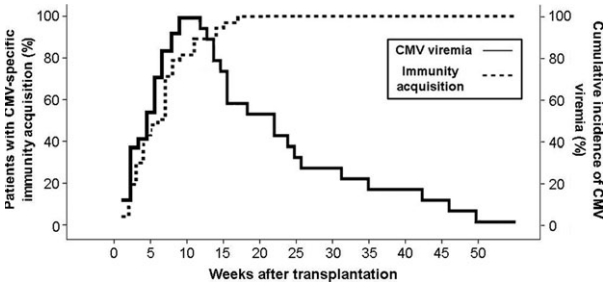

Figure 1 CMV replication and specific immune response. Evolution of CMV infection (continuous black line) measured by real-time PCR compared with detection of CMV-specific immune response (segmented line) by intracellular cytokine staining using flow cytometry. Progression between the acquisition of CMV-specific immune response and the decrease of the CMV viral load was compared by linear regression; r^2 = 0.941 with Pearson correlation = −0.971 (P-value = 0.01).

Characterization of the CMV-specific immune response

Viremic patients had levels of both $CD69^+CD4^+$ and $CD69^+CD8^+$ T cells significantly higher at the time of positive immune response compared with other time points (P = 0.01 and P = 0.03, respectively; Fig. 2), while no differences were found in nonviremic patients (Fig. 2). The percentage of $CD4^+$ and $CD8^+$ T cells secreting IFN-γ was significantly higher at the time of detection of CMV-specific immune response compared with other time points in both viremic and nonviremic patients (P = 0.001 and P = 0.02, respectively, Figure). Thus, regardless of time after the transplant for acquiring a positive immune response, no differences in the level of immune response were found between patients.

Timing of CMV-specific immune reconstitution and CMV replication incidence

To analyze timing for acquisition of a CMV-specific immune response and its influence on developing episodes of CMV replication, patients were grouped whether they acquired CMV-specific immune response before or after 6 weeks post-transplantation. Incidence of CMV replication was significantly lower in patients with positive CMV-specific immune response within 6 weeks after transplantation compared with patients that acquired a positive CMV-specific immune response after 6 weeks (33.3% vs. 89.5%; P-value = 0.007). Median percentage of $CD4^+$ and $CD8^+$ T cells secreting IFN-γ was also significantly higher in the group of patients that acquired a positive immune response before week six compared with after 6 weeks (median: 0.7 vs. 0.3 $CD8^+$ T cells/μl, P-value = 0.01; Fig. 3a and 3b), in addition to earlier secretion of IL-2 and IL-4, between weeks 2 and 6 (P-value = 0.02; data not shown).

Control of CMV infection by CMV-specific immune response

The 24 viremic patients developed a total of 76 CMV replication episodes. Seven (9.2%) of them, occurring after acquisition of a positive specific immune response, cleared without administration of treatment. These seven episodes consisted of small viral rebounds ranging from 86 to 2480 copies/ml with statistically significant reduction of viral load until day 21 (P-value = 0.02; Fig. 3c). Interestingly, the median percentage of IFN-γ secreting $CD8^+$ T cells was higher in these patients compared with those that received treatment (0.65% vs. 0.3%, respectively; P-value = 0.02).

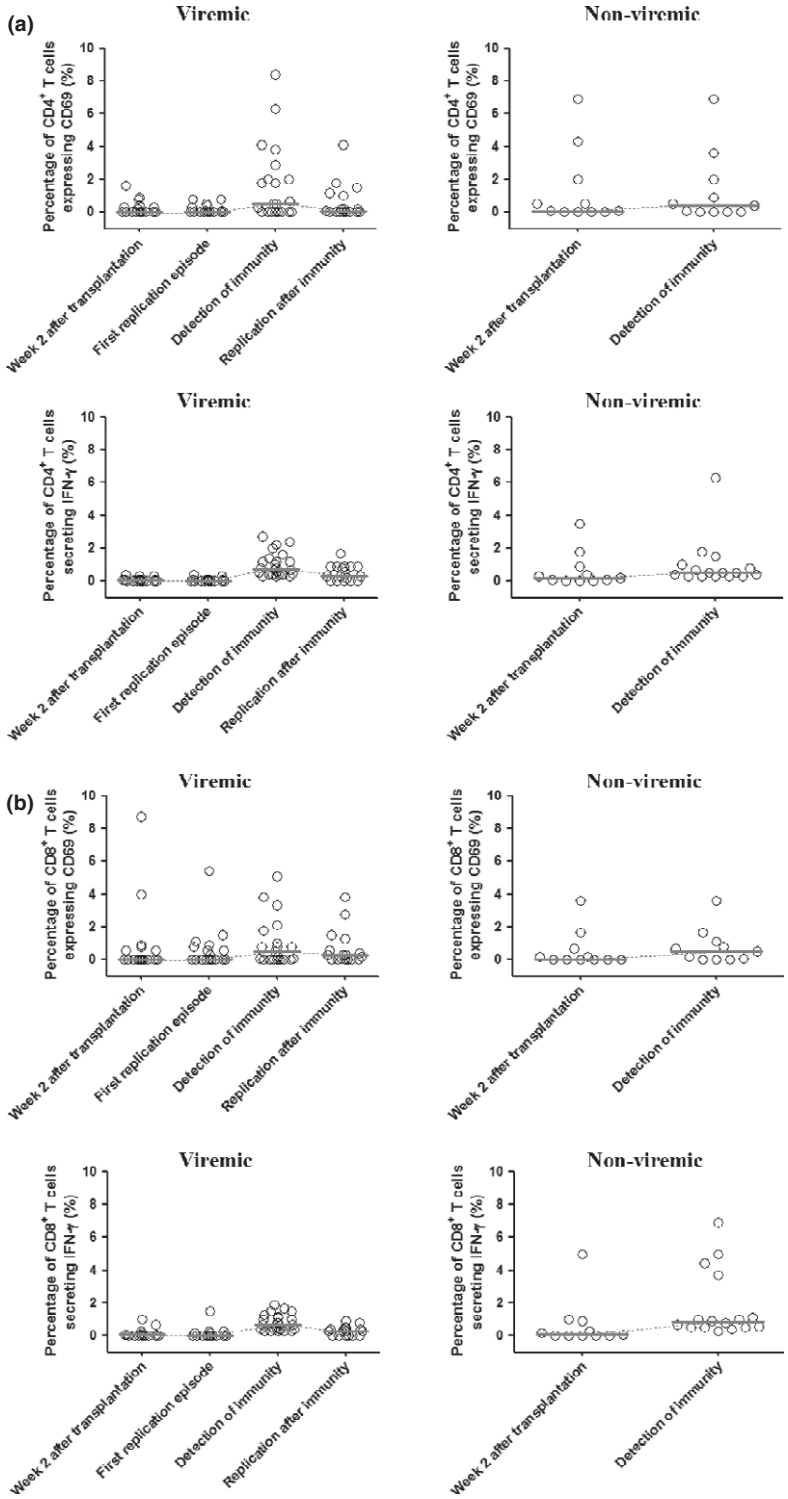

Figure 2 Surface marker and cytokines kinetics. CMV-specific immune response of $CD4^+$ (Panel a) and $CD8^+$ T cells (panel b) was evaluated in viremic and nonviremic patients at the indicated times after transplantation. Expression levels of early activation surface molecule CD69 and secretion of IFN-γ were compared in both $CD4^+$ and $CD8^+$ T cells between the different time points by Wilcoxon test. Viremic patients had levels of both $CD69^+CD4^+$ ($P = 0.01$) and $CD69^+CD8^+$ ($P = 0.01$) T cells significantly higher at the time of positive immune response compared with other time points. No significant differences were found in nonviremic patients. The percentage of $CD4^+$ and $CD8^+$ T cells secreting IFN-γ was significantly higher at the time of detection of CMV-specific immune response compared with other time points in both viremic ($P = 0.001$) and nonviremic patients ($P = 0.02$).

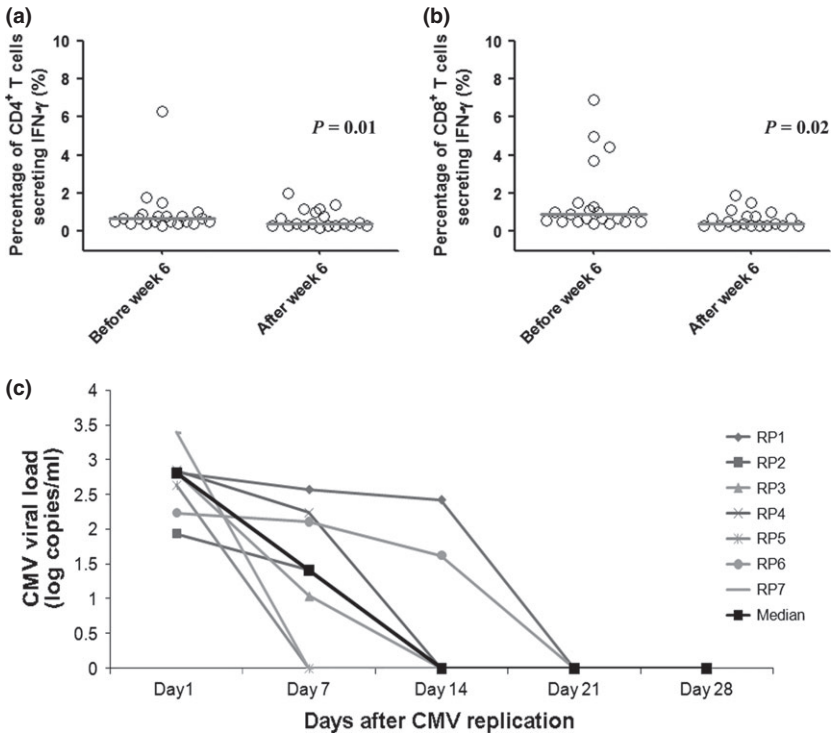

Figure 3 Level of the CMV-specific immune response after CMV infection and episodes of spontaneous controlled replication episodes. Patients with positive CMV-specific immune response within 6 weeks post-transplant reached significantly higher levels of INF-γ expressing $CD4^+$ (a) and $CD8^+$ (b) T cells than patients acquiring CMV-specific immune response after week six (*P*-value = 0.01). (c) Evolution of the viral load of the CMV replication episodes occurring after the acquisition of the immune response that were spontaneously cleared without treatment administration. The asterisks represent the statistically significant reduction of viral load at the different time points compared with day one (Wilcoxon test, *P*-value = 0.02). The black line represents the median viral load values at the different time points. RP, replication episodes.

$CD8^+$ T-cell chimerism analysis

Most of the patients achieved complete donor chimerism (95–100%). In addition, $CD8^+$ T-cell subpopulation was specifically investigated for chimerism after acquisition of CMV immune response and it was of complete donor origin also in 94.4% (34/36) of patients.

Risk factors for delayed CMV-specific immune reconstitution

Described risk factors [11,18,31,32] for delayed immune reconstitution were analyzed. In the univariate analysis, positive CMV serostatus of the recipients (OR = 13.2, CI 95% [16–101.9], *P*-value = 0.01), umbilical cord blood as a source of stem cells (OR = 10.5, CI 95% [1.1–20.2]; *P*-value = 0.03), and use of CsA plus MMF as GVHD prophylaxis (OR 4.8, CI 95% [1.04–22.1]; *P*-value = 0.04) were associated with delayed immune reconstitution (Table 2). In the multivariate analysis, no factor showed significant differences.

Risk factors for viral replication

Previously described risk factors for developing CMV infection [4,9] were also analyzed and compared between viremic and nonviremic patients. In the univariate analysis, the recipients pretransplant CMV-positive serostatus was associated with higher incidence of CMV replication (75% in viremic vs. 25% in nonviremic patients, (OR = 31.1, CI 95%; [13.4–55.2] *P*-value = 0.01). Delayed immune reconstitution after

Table 2. Risk factors for delayed CMV-specific immune reconstitution.

Risk factor	OR	CI 95%	*P*-value
R+/R−	13.2	16–101.9	0.01
D+R+/D−R+	1.93	0.38–9.64	0.4
UCB/other sources	10.5	1.1–20.2	0.03
CsP/MMF	4.8	1.04–22.1	0.04

CI 95%, confidence interval of 95%; R, CMV serostatus of the recipient; D, CMV serostatus of the donor; +, positive; −, negative; UCB, umbilical cord blood as stem cell source; CsP, cyclosporine; MMF, mofetil mycophenolate; OR, odds ratio.

week six was also associated with increased risk for viral replication (OR = 13.3 [CI 95%; 2.3–76.4]; *P*-value = 0.004). In the multivariate analysis, also both receptor pretransplant CMV-positive serostatus (OR = 8.3, CI 95% [1.3–51.9], *P*-value = 0.02) and CMV-specific immune response 6 weeks after transplantation (OR 27.2, CI 95% [2.2–328.7]; *P*-value = 0.009) persisted as risk factors for viral replication.

Timing of immune reconstitution and clinical outcomes

Survival was related with the timing of acquiring a positive CMV-specific immune response. One year overall survival was higher, although no statically significant, in patients with early (within 6 weeks post-transplantation) positive immune response, compared to patients with later (after 6 weeks) immune response (72%; CI: 0.53–0.96 vs. 42%; CI: 0.24–0.71; $P = 0.07$; Fig. 4a). This prognostic feature of early immune reconstitution also persisted as a trend for patients with cord blood graft and unrelated donor transplants (data not shown).

In addition, 1 year overall survival was significantly higher in patients with no CMV replication episodes compared with patients with at least one replication episode 41% (80% vs. 41% *P*-value = 0.02; Fig. 4b).

Furthermore, in patients experiencing CMV replication, 1-year transplant-related mortality was 33% (CI; 0.15–0.52), while no death occurred (CI: 0.05–0.34) in the group of patients with no CMV replication episodes (*P*-value = 0.01). Conversely, there was no difference in mortality caused by progression of the fundamental disease between viremic and nonviremic patients at 1 year post-transplantation.

Seven (14%) patients developed post-transplant end-organ CMV disease at a median of +77 days (range: 25 to 287 days). All cases were histologically confirmed and the sites were stomach ($n = 3$), colon ($n = 3$), and retina ($n = 1$). None of the deaths were caused by CMV end-organ disease. The percentage of INF-γ^+CD8$^+$ T cells in patients that developed CMV disease was lower than in patients with no CMV disease (median of 0.3% vs. 0.5%, respectively; $P = 0.02$).

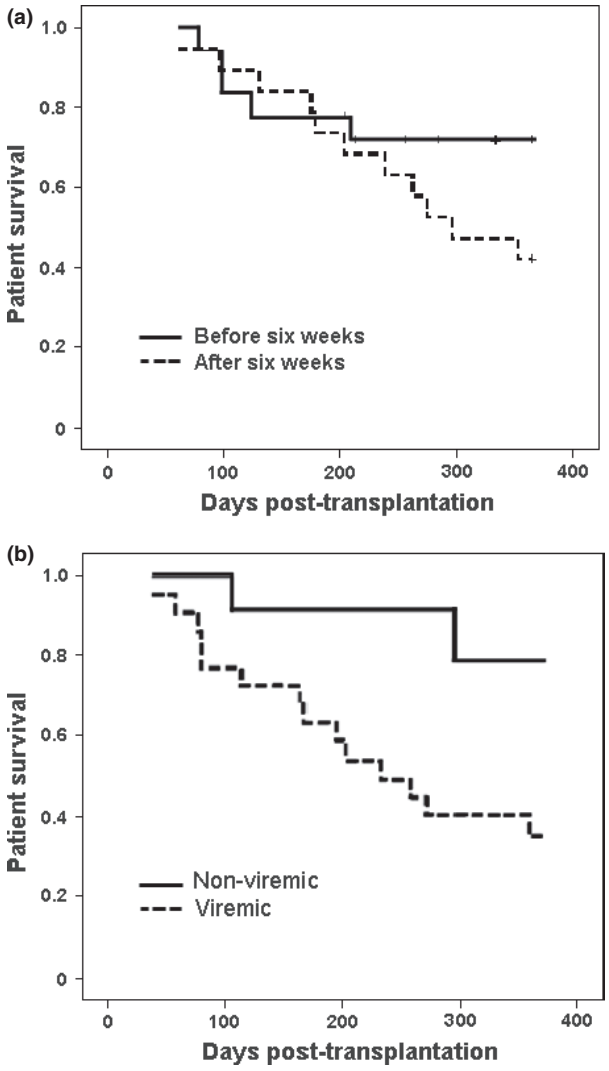

Figure 4 Patient survival. (a) One year overall survival for patients acquiring CMV-specific immune response either before (0.72; CI 95%; 0.536–0.962) or after 6 weeks (0.42; CI 95%; 0.249–0.713) post-transplantation. Differences in survival were compared by a log-rank test (*P*-value = 0.07). (b) One year overall survival for viremic (0.41) and nonviremic (0.80) patients. Differences in survival were compared by a log-rank test (*P*-value = 0.02).

Discussion

Despite improvement of antiviral therapy, reactivation of CMV remains an important clinical problem following hematopoietic stem cell transplantation [1,4,15,27]. Recipients at higher risk for CMV replication have reduced survival compared with recipients at lower risk [20]. It remains an elusive goal to define easy identifiable parameters of the CMV-specific immune response that allow to predict individual risk for CMV replication and its impact on clinical outcomes such as survival or transplant-related mortality.

This study prospectively focused on timing, kinetic, and magnitude of the CMV-specific T-cell immune response after HSCT and its ability to predict CMV replication and overall survival. Our results suggest that patients with a quick positive CMV-specific immune response, within 6 weeks post-transplantation, have reduced incidence of CMV replication. In fact, only one-third of patients that developed CMV-specific immune response within 6 weeks after transplantation experienced CMV replication compared with 90% of patients with a delayed immune response. Our results are in consonance with previously published results, although its biological significance and

clinical utility remain controversial [13,20,33–36]. These controversies may be explained by differences in the immune recovery after HSCT between pediatric and adult patients [15,35], different sources of HSC that may differ in the capacity of immune reconstitution [13,15,35,37] and aspects related with transplantation, such as conditioning or GVHD prophylaxis that may influence the post-transplant recovery [13–15,35]. The influence of CMV-specific immune reconstitution on CMV replication after HSCT has been extensively studied in the last years; however, its reciprocal relationship has not been completely clarified [13,15,35,37]. Some evidences suggest that CMV infection of the recipient may act as a booster for donor-derived antigen-experienced T cells [38,39]. We found a strong inverse correlation between the decline on CMV replication and the increase of acquisition of a positive CMV-specific T-cell response. In addition, the early acquisition (within the first 6 weeks post-transplant) of CMV-specific immune response also correlated with a much lower incidence of CMV replication. The biological and clinical relevance of a CMV-specific immune reconstitution is emphasized by the finding that 9% of patients with early immune reconstitution spontaneously controlled viral replication with no need of antiviral treatment which also correlated with a higher percentage of IFN-γ positive $CD8^+$ T cells. Our results are in line with a pilot study of intervention strategy, where HSCT patients with CMV-specific T-cell immunity recovery did not receive antiviral treatment after discontinuing prophylaxis and none of them developed CMV end-organ disease [14].

Several variables have been postulated as risk factors for delayed immune recovery after SCT. Lilleri *et al.* in a study performed in 57 patients found that total body irradiation in the conditioning regimen was positively related with higher CMV-specific $CD8^+$ T-cell response at day +30 [15]. We found that factors such as the CMV-positive serostatus of the recipient, umbilical cord blood graft as a source of stem cells, and use of CsA plus MMF as GVHD prophylaxis were associated with delayed immune reconstitution in the univariate analysis. In agreement with others [8,9,11,18], we also found that the pretransplant positive serostatus of the recipient was a risk factor for viral replication. In addition, we found that a delayed (after 6 weeks post-transplantation) CMV-specific T-cell reconstitution was also a risk factor for viral replication.

Our results also suggest that early immune reconstitution after transplantation has an impact in clinical outcomes demonstrated by an increase in survival cumulative probability. This difference in survival, although not reaching statistical significance, is clinically relevant. Based on timing of specific CMV immune response, we identified two subgroups of SCT recipients with different patterns of CMV replication and different main clinical outcomes. We speculate that management of CMV infection, prevention, and treatment should be different for both groups. In asymptomatic patients with early and robust post-transplantation positive CMV-specific immune response, immune surveillance may be enough to predict the control of viral replication, while preemptive antiviral treatment might be reserved for patients with higher viral loads or maintained viral replication. In patients with delayed immune reconstitution, preemptive antiviral therapy may not be optimal as it does not avoid indirect mortality related with CMV effects and antiviral-related toxicity and myelosuppression, suggesting that these patients may be better candidates for interventions of adoptive T-cell transfer immunotherapy. In these cases, repeated courses of antiviral treatment may be required to temporarily control viral infection. Adoptive transfer of virus-specific T cells generated and expanded *in vitro* may be beneficial for these patients [3,40].

These findings may indicate that future ways of immunotherapy to assist CMV-specific immune response reconstitution may enhance clinical efficacy [37]. It has also been suggested that routine immunologic monitoring will be helpful for guiding virologic assessment and therapeutic decisions in SCT recipients [15,35].

The limitation of our study is the relative small number of patients included; however, it was enough for achieving significant results.

In conclusion, our results suggest that the timing of the acquisition of CMV-specific immune response identifies two groups of HSCT recipients, with different patterns of CMV replication and clinical outcomes. This implies a difference in biological meanings of the post-transplant CMV infection, thus the ultimate cause of such a pattern needs to be determined.

Authorship

FC-V, OJB-H, IG-A, JRG-L: data collection and analysis, writing and final approval of the manuscript. MA-G, JMC, AU-I: provided patient care, data analysis, writing and final approval of manuscript. IE: provided patient care, conception and design, data collection and analysis, financial support, writing and final approval of manuscript. PP-R: conception and design, data collection and analysis, financial support, writing and final approval of manuscript.

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