Prospective simultaneous quantification of human cytomegalovirus-specific CD4⁺ and CD8⁺
T-cell reconstitution in young recipients of allogeneic hematopoietic stem cell transplantation

Short title: HCMV-specific T-cell reconstitution after HSCT

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Abstract

We investigated immune reconstitution against human cytomegalovirus (HCMV) in 57 hematopoietic stem cell transplantation (HSCT) recipients, aged 1-24 years, through a novel method combining T-cell stimulation by HCMV-infected autologous dendritic cells with simultaneous cytometric quantification of HCMV-specific, IFNγ-producing CD4⁺ and CD8⁺ T-cells. Lymphoproliferative response (LPR) to HCMV antigens was also determined. Patients were stratified into 2 groups according to HCMV serostatus, comprising 39 HCMV-seropositive (R⁺), and 18 HCMV-seronegative (R⁻) patients transplanted from a seropositive donor, respectively. Recovery of both HCMV-specific CD4⁺ and CD8⁺ T-cell immunity occurred in all 39 R⁺ patients within 6 months and in 6/18 (33%) R⁻ patients within 12 months. In R⁺ patients, the median numbers of HCMV-specific CD8⁺ and CD4⁺ T-cells were significantly higher than those of healthy controls, starting from day +60 and +180, respectively. In R⁻ patients, the median numbers of HCMV-specific T-cells were consistently lower than in R⁺ patients. LPR was delayed as compared to reconstitution of IFNγ-producing T-cells. Patients with delayed specific immune reconstitution experienced recurrent episodes of HCMV infection. HCMV-seropositivity of young HSCT recipients is the major factor responsible for HCMV-specific immune reconstitution, irrespective of donor serostatus, and measurement of HCMV-specific T-cells appears useful for correct management of HCMV infection.
Introduction

Human cytomegalovirus (HCMV) infection and disease are still frequent complications of patients given hematopoietic stem cell transplantation (HSCT). Use of antiviral drugs, according to either the prophylactic or the pre-emptive therapy approach, has significantly reduced morbidity and mortality. However, both approaches have disadvantages, such as occurrence of late HCMV disease for prophylaxis and need of strict virologic monitoring for pre-emptive therapy.

In humans, both HCMV-specific CD4+ and CD8+ arms of the T-cell immune response must be regenerated after HSCT in order to obtain long-term protection against HCMV reactivation and disease. The identification of the immunodominant peptides of the 2 major antigenic viral proteins pp65 and p72 in combination with the use of HLA-peptide tetramer technology and flow cytometry has allowed to better define the kinetics and magnitude of peptide-specific CD8+ T-cell response during immune reconstitution. In addition, it was shown that CD4+ T-cells are essential for sustained recovery of CD8+ T-cells. While tetramer technology does not detect the functional activity of T-cells, use of direct or autologous dendritic cell (DC)-mediated stimulation of T-cells by peptides deriving from pp65 and p72 in combination with cytokine flow cytometry (CFC), can identify the functional status of CD4+ and CD8+ T-cells recognizing specific peptides. In addition, the introduction of the enzyme-linked immunospot (ELISPOT) assay in combination with HLA-peptide tetramer staining, as well as the direct coupling of epitopic peptide stimulation with tetramer staining, has improved the understanding of the protective activity of HCMV-specific T-cells.

Through a novel method based on the simultaneous quantification of HCMV-specific CD4+ and CD8+ T-cells, we studied: i) virus-specific immune reconstitution in a population of young patients given HSCT; ii) the comparative kinetics of recovery of IFN-γ-producing CD4+ and CD8+ T-cells, as well as the lymphoproliferative response (LPR) to HCMV antigens; iii) the correlation between occurrence of single or recurrent episodes of HCMV infection and virus-specific immune reconstitution; iv) the number of HCMV-specific CD4+ and CD8+ T-cells critical for protection against HCMV infection; v) HSCT-related factors influencing immune reconstitution.
Patients and methods

Patients

From May 2003 through September 2005 a total of 61 patients (median age 9 years, range 1-24) undergoing allogeneic HSCT were evaluated for HCMV-specific T-cell reconstitution. The characteristics of the 61 patients are reported in Table 1. Four seronegative patients (R−) receiving the graft from a seronegative donor (D−) were excluded from the analysis. The ethics committee of Policlinico San Matteo approved the study, and patients or their parents gave written informed consent to the study.

Virologic follow-up

HCMV infection/reactivation was considered detection by any assay of HCMV in blood in the absence of clinical manifestations or organ function abnormalities, while HCMV disease was defined as either systemic or local HCMV infection, associated with clinical symptoms and/or organ function abnormalities.18

Patients were monitored for HCMV reactivation for 6-12 months after HSCT; they were randomised to be assigned to monitoring of HCMV reactivation by either the antigenemia or the DNAemia assay, according to methods previously described.19,20 In the antigenemia arm, patients were treated upon first detection of either 2 or more pp65-positive leukocytes or upon first confirmed positivity, when a single positive cell was detected. Therapy was stopped upon 2 consecutive negative results. Relapse episodes were treated similarly.21 In the DNAemia arm, patients were treated when reaching a cut-off of 10,000 DNA copies/ml whole blood, while treatment was stopped after 2 consecutive tests in which less than 1,000 DNA copies/ml were detected. Relapse episodes were treated similarly. Transient, self-resolving infection was defined by either a negative antigenemia test on the second control after a positive result or by repeated DNAemia values below 10,000 DNA copies/ml.

In addition, viremia, as well as donor/recipient HCMV serostatus, were evaluated according to previously reported methods.22,23

Pre-emptive therapy of HCMV infection was based on administration of intravenous ganciclovir (5 mg/kg twice a day). Ganciclovir was replaced by foscarnet (90 mg/kg twice a day) in case of ganciclovir-induced neutropenia or increasing viremia levels.
Patient treatment

All patients were given a fully myeloablative preparative regimen. Graft-versus-host disease (GvHD) prophylaxis consisted of cyclosporine-A (Cs-A) alone or associated to a short course of methotrexate for patients receiving the allograft from an HLA-identical sibling, whereas patients transplanted from an unrelated donor received anti-lymphocyte globulin (ALG) before HSCT in addition to Cs-A and short-term methotrexate. T-cell depletion of the graft was performed in patients given HSCT from a haplo-identical relative through positive selection of CD34+ G-CSF-mobilized peripheral blood cells. Acute GvHD was treated with steroids as first-line therapy (methylprednisolone 2 mg/Kg/day), while patients with steroid-resistant disease were treated with extracorporeal photochemotherapy.24

Immunologic follow-up.

HCMV-specific CD4+ and CD8+ T-cells were simultaneously quantified by a novel method based on use of autologous, monocyte-derived, HCMV-infected immature DC (iDC) as previously described.17 Briefly, following in vitro generation from peripheral blood mononuclear cells (PBMC),25 iDC were infected for 24 hours with an endotheliotropic and leukotropic strain of HCMV (VR1814), as previously reported.26,27 HCMV-infected iDC were then co-cultured overnight with autologous PBMC at a ratio of 1:20 in the presence of 10 µg/ml brefeldin A (Sigma, St. Louis, MO) to prevent cytokine release. Finally, PBMCs were tested for the frequency of HCMV-specific CD4+ and CD8+ interferon-γ (IFN-γ)-producing T-cells by the CFC assay.

Absolute CD3+CD4+ and CD3+CD8+ T-cell counts were determined on heparinized peripheral blood samples by direct immunofluorescence flow cytometry (Beckman Coulter Inc, Fullerton, CA). The total number of HCMV-specific CD4+ and CD8+ T-cells was calculated by multiplying the percentages of HCMV-specific T-cells positive for IFN-γ by the relevant absolute CD4+ and CD8+ T-cell counts. Based on results obtained by testing a series of HCMV-seropositive and HCMV-seronegative healthy blood donors, “responders” (i.e. patients in whom virus-specific immunity is present) were subjects with HCMV-specific CD4+ or CD8+ T-cells greater than 0.4/µl blood.17

LPR against HCMV was measured as previously reported.28 Cut-off for LPR was considered a stimulation index ≥3.0.
All patients were monitored for at least 6 months and 48 of them completed the 1-year follow-up. Of the remaining 13 patients, 2 died for transplant-related causes and 5 experienced leukemia relapse, while 6 are still in follow-up. Thirty-three HCMV-seropositive HSCT donors were taken as controls.

**Statistical analysis**

Data were analysed as of September 15, 2005.

Patient-, disease- and transplant-related variables were expressed as median and range or as percentage, as appropriate. The following variables were analyzed for their potential impact on HCMV-specific immune reconstitution: patient/donor age (the median was taken as cut point), T-cell depletion of the graft, ALG administration, occurrence of acute GvHD (0-I vs II-IV grades), need of steroid therapy, underlying disease (malignant vs non-malignant disorders), conditioning regimen (total body irradiation-based vs chemotherapy-based), donor type (unrelated donor vs HLA-identical sibling), HCMV donor serostatus (D+ vs D-), stem cell source (bone marrow vs peripheral blood), and type of monitoring (DNAemia vs antigenemia).

Differences between medians were compared by using the Mann-Whitney U test for unpaired data.

Curves of percentage of patients showing HCMV infection or HCMV-specific immune response in the first year after transplantation in the different groups of HSCT recipients were calculated and expressed as cumulative incidence, as reported, and compared by the log-rank test. P values lower than 0.05 were considered statistically significant. P values from 0.05 to 0.1 were considered statistically non-significant, but reported in detail. All variables with a P value less than 0.5 in univariate analysis were included in a multivariate analysis performed using the Cox-proportional hazard regression model. Receiver-operator curves (ROC) analysis was performed to identify levels of HCMV-specific CD4+ and CD8+ T-cells protective against HCMV infection.
Results

Incidence of HCMV infection and T-cell immune reconstitution in HCMV-seropositive and HCMV-seronegative HSCT recipients (R⁺ and R⁻)

None of the 4 D⁻/R⁻ patients experienced HCMV infection, and, as expected, they did not show a specific immune response during the 12 months after transplantation.

The cumulative incidence of HCMV infection/reactivation was significantly higher (p<0.01) in R⁺ (n=39) as compared with R⁻ (n=18) patients during the first year after transplantation (Fig. 1A). This higher incidence of virus reactivation was observed in R⁺ patients irrespective of the donor serological status. Only 6 R⁺ patients developed viremia. No patient developed HCMV disease.

In parallel, the cumulative incidence curves show that a significantly higher number (p<0.01) of R⁺ patients reconstituted (i.e. showed a specific T-cell number greater than 0.4 cells/µl blood) both CD4⁺ (Fig. 1B) and CD8⁺ (Fig. 1C) HCMV-specific T-cells as compared with R⁻ patients. More than 80% R⁺ patients reconstituted HCMV-specific T-cells within the first three months and all within 6 months after transplantation. By contrast, only 33% R⁻ patients developed specific immunity within the first year after transplantation, although their donor was HCMV seropositive.

A similar, although delayed, difference (p=0.004) between R⁺ and R⁻ patients was observed with regard to appearance of LPR to HCMV antigens (Fig. 1D). In fact, only 60% R⁺ patients reconstituted LPR within 6 months, while 15% of R⁺ patients were still unresponsive after one year.

As shown in Fig. 2A and B, the total CD4⁺ T-cell count increased over time after transplantation in both R⁺ and R⁻ patients, although it remained significantly lower than that of controls. The total CD4⁺ T-cell count was comparable in R⁺ and R⁻ patients at days +30 and +60, then becoming significantly higher (p<0.05) in R⁺ patients from day +90 onwards.

In the R⁺ group, the median number of HCMV-specific CD4⁺ T-cells was lower than that of controls at day +30, comparable at day +60 and day +90, and significantly higher from day +180 onwards (Fig. 2C). The median number of HCMV-specific CD4⁺ T-cells in the R⁻ group of patients was consistently lower than those of both controls and R⁺ patients during the entire first year after transplantation (Fig. 2 D).

The total count of CD8⁺ T-cells equaled that of controls at day +60 in R⁺ (Fig. 3A), while in R⁻ patients it remained lower than that of both controls and R⁺ patients for the entire follow-up
period (Fig. 3B). In R⁺ patients, the median number (Fig. 3C) of virus-specific CD8⁺ T-cells was significantly greater than that of controls, starting from day +60, with no significant further increase over time. By contrast, in R⁻ patients, the median number of virus-specific CD8⁺ T-cells was always significantly lower than that found in both R⁺ patients and controls (Fig. 3D).

Factors influencing the kinetics of reconstitution of CD4⁺ and CD8⁺ IFN-γ-producing T-cells.

None of the factors analyzed influenced the recovery of HCMV-specific CD4⁺ immune response. Only the use of total body irradiation (TBI) positively correlated with better CD8⁺ HCMV-specific immune response at day +30, median time to virus-specific CD8⁺ T-cell detection being 34 days as compared to 55 days for patients not receiving TBI (p=0.021). This variable remained significant in multivariate analysis (data not shown). At the other time points, no variable influenced CD8⁺ HCMV-specific immune reconstitution.

Relationship of HCMV-specific T-cell reconstitution at day +60 and control of HCMV infection in R⁺ patients

No difference in both HCMV-specific CD4⁺ and CD8⁺ T-cell counts, evaluated at day +60, was found between patients (n=10) with HCMV infection resolving after a single antiviral drug course and patients (n=16) experiencing a self-resolving infection, as defined before (Fig. 4A and B). R⁺ patients with HCMV infection showed levels of virus-specific CD8⁺ T-cells higher than those of patients (n=8) with undetected infection (Fig. 4B). Patients with relapsing episodes of HCMV infection (n=5) requiring multiple treatment courses showed HCMV-specific CD4⁺ and CD8⁺ T-cell counts much lower than those of all other patients (Fig. 4A, and B).

The median absolute number of IFN-γ producing CD4⁺ and CD8⁺ T-cells in 39 R⁺ patients at 60 days after transplantation is reported in Table 2. Numbers were stratified according to the occurrence of HCMV infection (undetected, self-resolving, requiring a single course of treatment, relapsing and requiring multiple courses of treatment). Day +60 was chosen as it was the earliest time point permitting to identify a threshold in the number of specific T-cells conferring protection against recurrent HCMV reactivations. It can be speculated from our data that the emergence of 1 cell/µl for CD4⁺ and 3 cells/µl for CD8⁺ may confer protection against recurrent episodes of viral reactivation. Results of ROC analysis for definition of optimal cut-offs for protection from recurrent infection are reported in Table 3.
Discussion

We investigated the kinetics and magnitude of HCMV-specific T-cell immune reconstitution in young patients given HSCT by means of a novel methodology.\(^{17}\) Our approach provides a more comprehensive assessment of T-cell reconstitution as compared with methods exploiting stimuli given by single peptides or based on staining produced by tetramers, and has the following advantages: i) HLA restriction does not limit the applicability of the assay; ii) it takes advantage of the simultaneous expression on DC membrane of different viral proteins, including pp65 and p72; iii) the method may be used for simultaneous quantification of both HCMV-specific CD4\(^{+}\) and CD8\(^{+}\) T-cells by CFC, and allows a functional evaluation of T-cells. The LPR assay complemented our approach in that it provided an estimate of the capacity of virus-specific T-cells to proliferate in short-term cultures in response to viral antigens.

To our knowledge, this is one of the few prospective studies focusing on reconstitution of immune response against HCMV in pediatric patients given HSCT. In our study, we found that all R\(^{+}\) patients recovered HCMV-specific CD4\(^{+}\) and CD8\(^{+}\) T-cell immunity within 6 months after transplantation, whereas R\(^{-}\) patients, although receiving all HSCT from D\(^{+}\) donors, reconstituted HCMV-specific T-cell immunity only in 6/18 (33\%) cases within 12 months. These data suggest that in this cohort of patients immune reconstitution was mainly driven by the pre-transplant HCMV infection of the recipient, in turn facilitating post-transplantation viral reactivation. This conclusion is in keeping with previous reports suggesting that HCMV infection of the recipient may act as a booster for donor-derived, antigen-experienced, T-cells.\(^{31,32}\) The relevance of virus-specific immune reconstitution sustained by donor T-cells was recently emphasized in a large multicenter European study showing that R\(^{+}\) patients receiving graft from unrelated D\(^{+}\) donor had an improved 5-year survival and a reduced transplant-related mortality (TRM) as compared with R\(^{+}\) patients transplanted from D\(^{-}\).\(^{33}\) Furthermore, seropositive patients transplanted from D\(^{-}\) have been reported to have an increased risk of developing high levels of antigenemia as compared to R\(^{+}\) patients given the allograft from a D\(^{+}\).\(^{34}\) In conclusion, in R\(^{-}/D^{+}\) pairs, early immune recovery appears to be supported by adoptive transfer of memory T-cells from donor to recipient, and HCMV infection may be crucial in favoring the expansion of memory T-cells.
The observation that, in our cohort, patients receiving graft from a seronegative donor or a T-cell depleted graft do not show a delay in HCMV-specific T-cell reconstitution raises the question of how a pathogen-specific immune response can develop in the absence of or after physical removal of memory T-cells. Previously published studies in patients given T-cell depleted HSCT or cord blood transplantation suggested that recipient-derived T-cell clones may also contribute to post-transplantation immune recovery. Thus, in some selected R⁺ patients early and sustained immune reconstitution may also be sustained by recipient T-cells, whose survival and expansion is triggered by HCMV reactivation. Development of a primary immune response, although in the context of a profound state of immune deficiency characterizing the post-transplantation period, may be advocated to explain the presence of HCMV-specific T-cells in R⁺ recipients transplanted from D⁻ donors. In this regard, children may have a better capacity than adults in developing anti-HCMV primary immune response after HSCT.

In R⁻ patients transplanted from D⁺ donors, in the absence of detectable post-transplantation HCMV infection, immune reconstitution does occur only in a minority of patients. An antigen-independent, cytokine-driven expansion of donor memory T-cells adoptively transferred with the graft can be taken into consideration for explaining immune reconstitution in these patients. Subclinical episodes of HCMV infection at sites other than blood may also have potentially contributed to the virus-specific immune reconstitution in R⁻ patients.

Due to the very strict virological monitoring and the early pre-emptive approach adopted, a few patients developed viremia and none suffered from HCMV disease. Thus, occurrence of relapsing infection requiring multiple courses of antiviral treatment was taken into account as an indicator of potentially severe HCMV infection. Results of ROC analysis seem to confirm that levels of CD4⁺ <1 cell/µl and of CD8⁺ <3 cells/µl are not protective against recurrent infection. In our study, repeated episodes of HCMV reactivation occurred in patients without a prompt recovery of HCMV-specific immunity. Lack of post-transplantation immune regeneration has been reported to be consistently associated with relapses of HCMV infection and development of HCMV disease. In these cases, repeated courses of antiviral treatment are required to temporarily control viral infection, and this may cause emergence of drug-resistant HCMV strains or may lead to myelosuppression. Adoptive transfer of virus-specific T-cells generated and expanded in vitro may be beneficial to these patients.
In our patients reconstituting cellular immunity, the magnitude of HCMV-specific CD8⁺ T-cell reconstitution exceeds that of CD4⁺ T-cell response, the number of virus-specific CD8⁺ T-cells being much higher than that found in controls and persisting during the entire follow-up period. This finding was already reported in previously published studies, showing in immunosuppressed individuals an increased presence of HCMV-specific CD8⁺ T-cells that may represent up to 50% of the total CD8⁺ T-cell compartment, remaining elevated for months or even years.\(^{41}\)

Our results support the previously published observation that when donors and recipients are HCMV seropositive, virus-specific CD4⁺ T-helper cells show the same reconstitution kinetics as CD8⁺ cytotoxic T-cells.\(^{42}\) This finding is also in agreement with previous studies indicating that patients showing LPR to HCMV antigen (attributed to CD4⁺ T-cell activity) were protected against HCMV recurrent reactivation and disease.\(^{12}\) As already observed in primary HCMV infection of immunocompetent subjects and pregnant women,\(^{43}\) we found that recovery of LPR to HCMV antigens is delayed as compared to reconstitution of CD4⁺ and CD8⁺ IFN-γ producing T-cells.

While no factor influenced HCMV-specific CD4⁺ T-cell recovery, TBI as part of the preparative regimen was the only variable enhancing virus-specific CD8⁺ T-cell reconstitution at day +30. The interpretation of this finding remains unclear. It is possible that the relatively low number of patients analyzed precluded the possibility of identifying the impact of other variables.

In conclusion, our results demonstrate that many R⁺ young patients given HSCT consistently reconstitute protective HCMV-specific immunity within 6 months. In the future, routine immunological monitoring will be helpful in guiding virological monitoring and therapeutic decisions in young HSCT recipients. Interventions with antiviral drugs could be limited in patients with T-cell immune response, while sustained lack of immune reconstitution could be compensated with interventions of adoptive T-cell transfer immunotherapy.
Acknowledgments

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Table 1. Characteristics of the 61 patients analyzed.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patient number (%)</th>
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</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>32/29</td>
</tr>
<tr>
<td>Median age at transplantation (range)</td>
<td>9 (1-24) years</td>
</tr>
<tr>
<td>Underlying disease</td>
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</tr>
<tr>
<td>malignant</td>
<td>37 (61)</td>
</tr>
<tr>
<td>non-malignant:</td>
<td>24 (39)</td>
</tr>
<tr>
<td>Stem cell source:</td>
<td></td>
</tr>
<tr>
<td>bone marrow</td>
<td>43 (70)</td>
</tr>
<tr>
<td>peripheral blood</td>
<td>14 (23)</td>
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<tr>
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<td>4 (7)</td>
</tr>
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<td>Donor type:</td>
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<tr>
<td>sibling</td>
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<tr>
<td>unrelated</td>
<td>27 (44)</td>
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<tr>
<td>haploidentical relative</td>
<td>12 (20)</td>
</tr>
<tr>
<td>Donor/recipient HCMV serostatus:</td>
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</tr>
<tr>
<td>D+/R+</td>
<td>28 (46)</td>
</tr>
<tr>
<td>D+/R−</td>
<td>11 (18)</td>
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<tr>
<td>D+/R−</td>
<td>18 (29)</td>
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<tr>
<td>D−/R−</td>
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<tr>
<td>Conditioning regimen</td>
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<tr>
<td>TBI based</td>
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<tr>
<td>non-TBI based</td>
<td>34 (56)</td>
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<td>GvHD prophylaxis:</td>
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<td>Administration of ALG:</td>
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<tr>
<td>II-IV</td>
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<td>Steroid therapy</td>
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<td>Yes</td>
<td>26 (43)</td>
</tr>
<tr>
<td>No</td>
<td>35 (57)</td>
</tr>
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CS-A, cyclosporine-A; MTX, methotrexate; ALG, anti-lymphocyte globulin; GvHD, graft-versus-host disease; TBI, total body irradiation.
Table 2. Median absolute number of HCMV-specific T-cells according to different types of HCMV infection in 39 R+ patients 60 days after transplantation.

<table>
<thead>
<tr>
<th>Type of HCMV infection</th>
<th>Median number (range) of HCMV-specific T-cells/µl</th>
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<tbody>
<tr>
<td></td>
<td>CD4⁺</td>
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<tr>
<td>Undetected</td>
<td>1.62 (0.91-15.78)</td>
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<tr>
<td>Self-solving</td>
<td>2.88 (1.07-82.85)</td>
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<tr>
<td>Treated</td>
<td>3.79 (1.60-15.01)</td>
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<tr>
<td>Relapsing</td>
<td>0.12 (0.01-0.76)</td>
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Table 3. ROC analysis for definition of optimal cut-offs for protection from recurrent infection.

<table>
<thead>
<tr>
<th>Cutoff (T-cells/µl)</th>
<th>CD4⁺</th>
<th></th>
<th>CD8⁺</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sens %</td>
<td>Spec %</td>
<td>PPV %</td>
<td>NPV %</td>
</tr>
<tr>
<td>0.4</td>
<td>100</td>
<td>80</td>
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<td>100</td>
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<td>83</td>
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<td>5.0</td>
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<td>17</td>
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<tr>
<td>7.0</td>
<td>26</td>
<td>100</td>
<td>100</td>
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<td>10.0</td>
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Sens, sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value.
Figure 1. Probability of developing HCMV infection (A), of reconstituting HCMV-specific CD4+ (B) and CD8+ (C) T-cell immunity (i.e. corresponding to a specific T-cell number greater than 0.4 cells/µl blood) and of restoring LPR to HCMV (D) in the 2 groups of HCMV R+ and R− HSCT recipients.
**Figure 2.** Absolute number of total and of HCMV-specific CD4+ T-cells in the 2 groups of HCMV R+ (A, and C) and R− (B, and D) transplanted patients during the first year after transplantation.
Figure 3. Absolute number of total and of HCMV-specific CD8+ T-cells in the 2 groups of HCMV
R+ (A, and C) and R− (B, and D) transplanted patients during the first year after transplantation.
Figure 4. Relationship between absolute number of HCMV-specific CD4+ and CD8+ T-cells (A, and B) and different types of HCMV infection in HCMV R+ HSCT patients at 60 days after transplantation.
Prospective simultaneous quantification of human cytomegalovirus-specific CD4⁺ and CD8⁺ T-cell reconstitution in young recipients of allogeneic hematopoietic stem cell transplantation

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