

ORIGINAL ARTICLE

Patients at high risk for CMV infection and disease show delayed CD8 + T-cell immune recovery after allogeneic stem cell transplantation

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Human cytomegalovirus (CMV) is a major cause of death after transplantation. The frequency of pp65-specific T cells was examined in 38 HLA-A2 + stem cell recipients during the first year after transplantation. Patients were divided into four groups based on donor/recipient serostatus: d +/r + (n = 17), d +/r - (n = 7), d -/r + (n = 9) and d -/r - (n = 5). Peripheral blood mononuclear cells were stimulated with the CMVpp65 peptide NLVPMVATV, and the specific T-cell frequency was assessed by interferon gamma (IFN- γ) ELISPOT assay. Responding T cells were characterized by flow cytometry revealing a terminal differentiated effector phenotype. Surveillance of CMV infection was carried out by real-time polymerase chain reaction (n = 26) or immunofluorescence (n = 12). Infection was present in 7/9 d -/r + high-risk patients, and CMV disease occurred exclusively in this group with delayed or absent virus-specific T-cell recovery. In contrast, 16/24 intermediate-risk patients showed CMV-specific T cells. Our data suggest that CMV infection and disease rates are elevated in high-risk patients with delayed CMV-specific T-cell immune reconstitution and lower in those with early recovery of T-cell immunity. We recommend preferring CMV seropositive donors for CMV seropositive recipients, as this should lead to durable CMV-specific T-cell responses soon after transplantation with consecutive protection from CMV disease.

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Introduction

Owing to the long period of immunodeficiency after allogeneic hematopoietic stem cell transplantation (HSCT), human cytomegalovirus (CMV) infections remain one of the most important factors influencing morbidity and mortality during the first year after HSCT. Numerous well-designed clinical trials have demonstrated the major impact of recipient seropositivity in the early phase after transplantation.^{1–3} Furthermore, T-cell-depleted grafts and immunosuppressive drugs for conditioning and therapy or prophylaxis of acute graft-versus-host disease (GVHD) have been shown to influence the incidence of CMV infections early after HSCT.^{1,3–6} Although the CMV infection rate can be reduced by pre-emptive antiviral therapy,³ this strategy often leads to increased toxicity and even morbidity through impaired hematological recovery and other severe side effects like renal insufficiency. Moreover, CMV infections still occur during the late phase^{1,4,6–9} after discontinuing close antigenemia monitoring or prophylactic antiviral treatment. Risk-adapted treatment based on immunological variables such as CMV-specific T-cell recovery could help to improve the standard strategies mentioned. Although healthy immunocompetent hosts can prevent (re)activation of CMV disease by protective CD4 + and CD8 + T-cell responses, patients with a compromised immune system are prone to developing CMV infections.^{10,11} Virus-specific memory T cells in a stem cell graft from a seropositive donor should enable the recipient to develop an immune response strong enough to control and subsequently destroy the virus. If, on the other hand, a CMV seropositive patient receives a transplant from a seronegative donor without CMV-specific memory T cells, the reconstitution of CMV-specific T-cell immunity will be gradual and prolong the patient's high risk for CMV infection and disease.^{3,6,12} To confirm this hypothesis and investigate CMV-specific T-cell recovery in relation to the occurrence of infection and disease in patients grouped by their CMV serostatus, we prospectively examined the frequency of peripheral pp65-specific T cells in 38 HLA-A2 + stem cell recipients. After peripheral blood mononuclear cells (PBMC) were stimulated with the

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CMV-derived peptide NLVPMVATV, the frequency of pp65-specific T cells was assessed by interferon gamma (IFN- γ) ELISPOT assay at defined intervals after HSCT. In patients with high frequencies of CMV-specific T cells, the responding cells were selected by a cytokine-capture assay and further characterized for their phenotype by flow cytometric analysis.

Materials and methods

Blood samples and patient characteristics

Our study enrolled 38 HLA-A2+ patients who presented to the University Hospitals in Leipzig and Berlin with different hematological malignancies (acute myeloblastic leukemia, myelodysplastic syndromes, acute lymphoblastic leukemia, non-Hodgkin's lymphoma and chronic myeloid leukemia) between 2000 and 2005. Their characteristics are summarized in Table 1. They had a median age of 49 years (range 17–66 years). Thirty patients were transplanted from a matched unrelated donor and eight from a matched related donor. Patients were conditioned according to standard protocols and received total-body irradiation/cyclophosphamide ($n=16$), fludarabine/total-body irradiation ($n=8$) or BU/Cy ($n=7$). According to standard procedure protocols, antithymocyte globulin (ATG) was additionally administered to 20/38 patients (15 patients with 'ATG Fresenius S' 15 mg/kg and five patients with thymoglobulin Sangstad 2 mg/kg for 3 days). Most of the patients ($n=27$) received GVHD prophylaxis with cyclosporin A and methotrexate. Targeted time points were 30, 60, 90, 120 and 180 days after HSCT. Samples were taken more often in patients with prominent T-cell responses. The median follow-up was 38 weeks (range 3–92 weeks after transplantation). All investigations were performed with fresh blood samples in order to avoid possible influences caused by repeated freeze-thaw cycles. PBMC were isolated by Ficoll-Paque (Biochrom, Berlin, Germany) density gradient centrifugation.

Peptides

Lymphilized peptides (Affina Immuntechnik GmbH, Berlin, Germany) were dissolved in dimethyl sulfoxide at 40 mg/ml, diluted in phosphate-buffered saline (PBS) to a final concentration of 2 mg/ml and stored at -20°C . The following HLA A*0201 binding peptides were synthesized: CMV_{65–73} NLVPMVATV,¹³ and, for the negative control, HIV-1_{476–484} ILKEPVHGV.¹⁴ Phytohaemagglutinin was used for the internal control.

IFN- γ ELISPOT assay

Nitrocellulose HA S45 plates (Millipore, Eschborn, Germany) were coated overnight at 4°C with anti-IFN- γ antibodies (clone 1-D1K, 10 $\mu\text{g}/\text{ml}$; Mabtech, Nacka, Sweden) in a carbonate/bicarbonate buffer and then washed six times with PBS. The remaining free-binding sites were blocked using AIM-V medium (GibcoBRL, Karlsruhe, Germany) with 5% inactivated human serum albumin (Sigma-Aldrich, Steinheim, Germany). PBMC ($1 \times 10^5/\text{well}$ in 100 μl) were incubated with 1 $\mu\text{g}/\text{ml}$ of

Table 1 Characteristics of allografted patients

	n
<i>Sex</i>	
Female	17
Male	21
<i>Median age, range</i>	
49, 17–66	
<i>Underlying disease</i>	
AML/MDS	19
ALL	7
CML	8
NHL	4
<i>Donor</i>	
Matched related	8
Matched unrelated	30
<i>Stem cell source</i>	
Peripheral blood stem cells	38
<i>Conditioning regimen</i>	
TBI/Flu	8
TBI/Cy	16
BU/Cy	7
Treo/Flu	5
Other	2
<i>GVHD prophylaxis</i>	
CSA/MTX	27
CSA/MMF	6
CSA	4
Other	1
<i>Grade of GVHD</i>	
I, II	22
III, IV	4
No GVHD	12
<i>Donor recipient CMV serostatus</i>	
-/- (Group 1)	5
+/+ (Group 2)	17
+/- (Group 3)	7
-/+ (Group 4)	9
<i>Risk group</i>	
Intermediate (+/+, +/-)	24
High (-/+)	9
Low (-/-)	5

Abbreviations: AML=acute myeloblastic leukaemia; BU=busulfan; CML=chronic myeloid leukemia; CMV=cytomegalovirus; CSA=cyclosporine A; Cy=cyclophosphamide; Flu=fludarabine; GVHD=graft-versus-host disease; MMF=mycophenolate mofetile; MTX=methotrexate; NHL=non-Hodgkin's lymphoma; TBI=total-body irradiation; Treo=treosulfan.

CMV and HIV-1 antigen-specific peptide in AIM-V medium for 16 h at 37°C . Assays were performed in triplicate. After removing cells, plates were incubated with a biotinylated secondary antibody against human IFN- γ and streptavidin-alkaline phosphatase (Mabtech, Nacka, Sweden). NBT/BCIP (ICN, Eschwege, Germany) colorization was carried out according to the manufacturer's instructions. Spots were counted using a BIOREADER 2000 image analyzer (BIOSYS, Karben, Germany). The frequencies of peptide-specific T cells were calculated by

subtracting the mean number of nonspecific IFN- γ spots in the control samples from the mean number of IFN- γ spots in the peptide-stimulated samples. An IFN- γ ELISPOT response was defined as 'positive' when the frequency was higher than 20 spots/100 000 cells after subtracting the control sample value.

IFN- γ secretion assay

IFN- γ secreted by antigen-specific cells was measured when the *ex vivo* frequency of IFN- γ -producing cells was >0.05% of 100 000 PBMC and when sufficient numbers of cells were available. Fresh PBMC were resuspended in Roswell Park Memorial Institute 1640 medium supplemented with 5% human albumin (Behring-Chiron Marburg, Germany). Cells were incubated for 16 h alone or in the presence of 40 μ g/ml of peptide. Cell surface detection of IFN- γ -secreting cells was performed by first labeling cells for 5 min at 4°C with an IFN- γ -specific high-affinity capture matrix (MiltenyiBiotec, Bergisch Gladbach, Germany), that is, a bispecific antibody-antibody conjugate directed against both CD45 and IFN- γ . The cells were subsequently transferred to a warmed-up medium at 37°C for 45 min to permit IFN- γ secretion; they were then washed and labeled with anti-PE microbeads (Miltenyi-Biotec, Bergisch Gladbach, Germany) for 15 min at 4°C. PE-labeled IFN- γ -positive cells were enriched in two rounds of positive selection by magnetic cell sorting (purified fraction) and immediately analyzed by flow cytometry.

Flow cytometric analysis

Immunophenotyping of selected IFN- γ -producing cells was carried out by three-color flow cytometry according to standard procedures. Briefly, 100 000 IFN- γ PE-conjugated cells per tube were marked by monoclonal antibodies to CD8, CD4, CD56, CD45RA, CD27, HLA-DR and CCR7. All antibodies except anti-CCR7 (R&D, Wiesbaden-Nordenstadt, Germany) were obtained from BD (Heidelberg, Germany). After adding the fluorescence-labeled antibody, cells were incubated for 15 min at 4°C and washed in PBS, pending analysis.

CMV infection and disease

CMV infection and disease (pneumonia, retinitis, hepatitis, gastroenteritis) were defined according to Ljungman *et al.*¹⁵ All our study patients had CMV monitoring by polymerase chain reaction (PCR) and/or indirect immunofluorescence. Monitoring was carried out twice a week during the infection phase. It was carried out three times a week in high-risk patients and those suffering from infections during or shortly after the neutropenic phase. Patients with CMV disease were submitted to follow-up and further diagnostic monitoring only in cases with persistent or recurrent clinical symptoms.

Patients with CMV infection and disease were treated with the antiviral drug ganciclovir until antigenemia or PCR copy numbers were below the detection limit in two consecutive blood samples. Nonresponders to ganciclovir, that is, those with increased viral loads despite antiviral therapy, were treated with foscarnet or cidofovir.

Immunoglobulins were used to treat lymphopenic or severely immunocompromised patients or poor responders to antiviral therapy.

Determination of CMV serostatus and detection of CMV DNA and antigen pp65 in clinical samples

CMV immunoglobulin G (IgG) serostatus was quantitatively determined using 'Enzygnost anti-CMV IgG' (Dade-Behring, Germany) or 'AxSYM CMV IgG' (Abbott, Illinois, USA). CMV-specific IgM was detected by 'CMV-IgM-ELA Test PKS' (Medac GmbH, Hamburg, Germany) or 'Vidas CMV-IgM' (bioMérieux, Nürtingen, Germany). All assays were carried out according to the manufacturer's instructions. Viral nucleic acid was isolated using the NucleoSpin Blood (50) kit (Macherey & Nagel, Düren, Germany). For the quantitative detection of viral DNA a fragment of the HCMV-UL123 gene was amplified by real time PCR with a sensitivity of 200 geq/ml. Antigenemia monitoring was performed with a commercially available assay (CINA Kit, Argene). Detailed information about the protocols and performance of both certified assays are available on request from the authors.

Statistical analysis

The Fisher's exact test was used to compare the rate of infection and disease in the d+/r+ and d-/r+ groups and to analyze a possible impact of ATG administration on the specific T-cell count in the intermediate-risk group (d+/r+). *P*-values below 0.05 were regarded as statistically significant.

Results

CMVpp65-specific T-cell recovery was prospectively assessed after pulsing PBMC with the CMVpp65 peptide NLVPMVATV by IFN- γ ELISPOT assay in 38 patients before HSCT and at defined times thereafter. Cells pulsed with the HIV peptide ILKEPVHGV served as a negative control. After stimulation with the HIV peptide, the frequency of IFN- γ positive cells was consistently below 20 spots/100 000 cells (mean 4, range 0–7 spots/100 000 cells), thus excluding unspecific stimulation.

CMV-specific T-cell response in the different risk groups

Patients were divided into different risk groups according to the donor/recipient CMV serostatus and subsequent risk of CMV infection and/or disease. Table 2 gives an overview about the number of T-cell responses in every group, their frequency and range within the first 16 weeks after allo HSCT. Figure 1 demonstrates the time course of peptide-specific T cells, Figure 2 the frequency of CMV infection and disease.

The low-risk group (d-/r-) consisted of five CMV seronegative patients with seronegative donors. CMV infection, disease or CMV-specific T cells were not observed in any of these patients (Figures 1 and 2).

Patients with a CMV seropositive donor were included in the intermediate-risk group regardless of their own CMV serostatus. The number of patients with CMV-specific T

Table 2 Frequency of CMVpp65-specific T cells in allogeneic transplanted patients

	Total n	Weeks after allo HSCT	No. of patients with CMVpp65- T-cell response	Range of spots/100 000 cells (mean)
Low risk d-/r-	5	d-1	0	9-20 (7)
		4-5	0	0-3 (1)
		8-10	0	0-7 (4)
		13-16	0	0-13 (3)
Intermediate risk d+/r+ and d+/r-	24	d-1	8	51-665 (135)
		4-5	11	29-797 (183)
		8-10	15	37-804 (343)
High risk d-/r+	9	13-16	16	27-1225 (551)
		d-1	5	0-97 (35)
		4-5	1	74
		8-10	0	3-19 (4)
		13-16	0	0-8 (3)

Abbreviations: CMV = cytomegalovirus; HSCT = hematopoietic stem cell transplantation.

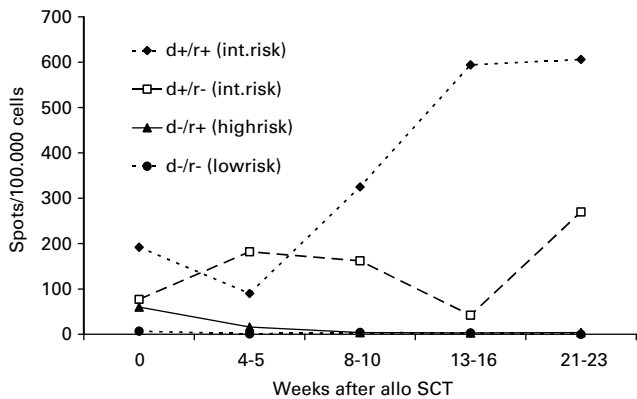


Figure 1 Time course of CMV-specific T cells in the four groups. It demonstrates the course of CMV-specific T-cell frequencies (mean values) in the four different risk groups during 23 weeks after allogeneic transplantation. Although high and stable frequencies of peptide-specific T cells appeared in most intermediate-risk patients (d+/r+ and d+/r-) soon after HSCT and lasted for the entire investigation period, no CMV-specific T-cell frequencies were detected in the high-risk patients. Seronegative patients allografted with a seronegative donor did not develop any peptide-specific T-cell responses to CMV.

cells increased soon after transplantation (Figure 1) from 11/24 patients (46%, 4-5 weeks after HSCT) up to 16/24 patients (67%, 13-16 weeks after HSCT). Of the remaining eight patients without a specific T-cell response after 4 months, four were not yet past day 120 after HSCT; two remained without a response even more than 1 year after HSCT; one patient died; and one developed weak but stable CMV-specific T-cell frequencies 9 months after HSCT (range 26-31, mean 28 spots/100 000 cells). Nine patients were monitored for 1 year after HSCT. Eight maintained a strong CMV-pp65 specific T-cell response ranging from 49 to 1022 spots/100 000 cells (mean 639).

In the group of CMV seropositive patients allografted with a CMV seronegative donor, 5/9 patients initially had pp65-specific T cells whereas only 1/9 patients had

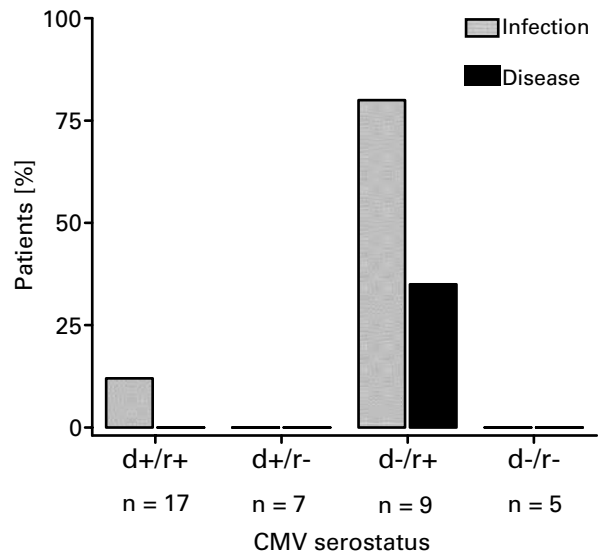


Figure 2 CMV infection and disease in patients after allo HSCT. It shows the percentage and number of patients with post-HSCT CMV infection and disease according to risk groups. CMV infection was detected in 7/9 (78%) patients from the high-risk group (d-/r+). Three of those seven patients suffered from severe CMV disease (33%). Only two of 17 intermediate-risk patients (d+/r+) (12%) developed CMV infection but showed no clinical symptoms of CMV disease.

CMV-specific T cells during the first month after transplantation. Specific T cells were not detectable in any of the patients at a later stage after allo HSCT (Figure 1).

CMV-specific T-cell immunity and frequency of CMV infection and disease

In total, 9/38 patients were affected by CMV infection and/or CMV related disease (Table 3). Although infection was assessed in 2/24 intermediate-risk patients, both did not develop CMV disease during the first year after HSCT (Figure 2). Subsequent ganciclovir treatment successfully aborted the infection. In both patients, T-cell responses started to increase when infection developed with stable frequencies at a relatively high level of up to 1328 spots/100 000 cells.

However, compared to the intermediate-risk group, a significantly higher proportion of high-risk patients developed CMV infection (7/9 patients, 78%; $P < 0.05$) or CMV disease (3/7 infected patients; 43%; $P < 0.05$; Figure 2).

Relevant and lasting CMV-specific T-cell immunity failed to develop in 8/9 patients (six infected patients). One of the patients with intermittent infection showed single and weak T-cell responses in 4/9 incoherent samples (mean 53, range 24-77 spots/100 000 cells) that did not correlate with frequent periods of reinfection.

None of the patients with CMV disease had detectable T-cell responses during the disease period. Only one of three patients reconstituted CMV-specific T cells 19-27 weeks after HSCT (mean 366, range 140-798 spots/100 000 cells). However, no CMV-specific T cells were detectable either during the phase of frequent reinfections within the first 19 weeks after HSCT or during the onset of disease 25

Table 3 Frequency of CMV infection and disease in nine allogeneic transplanted patients

	<i>Risk group</i>	<i>CMV infection (days after allo HSCT)</i>	<i>CMV disease (days after allo HSCT)</i>	<i>Diagnostic/therapeutic interventions and medication</i>
Patient 1	d+/r+	d+41	—	Ganciclovir
Patient 2	d+/r+	d+27	—	Ganciclovir
Patient 3	d-/r+	d+13	Pneumonia d+16	Bronchoscopy, ganciclovir
Patient 4	d-/r+	d+14	Pneumonia d+22	Bronchoscopy, transient artificial respiration ganciclovir, foscavir, cidofovir
Patient 5	d-/r+	d+34	Stomach and liver d+49, retinitis d+213	Gastroenteroscopy, vitrectomy, ganciclovir
Patient 6	d-/r+	d+22	—	Ganciclovir
Patient 7	d-/r+	d+18	—	Ganciclovir
Patient 8	d-/r+	d+27	—	Ganciclovir
Patient 9	d-/r+	d+17	—	Ganciclovir

Abbreviations: CMV = cytomegalovirus; HSCT = hematopoietic stem cell transplantation.

days thereafter. After this patient started to show increasing specific T-cell frequencies in the 19th week, only two more single positive results were obtained for the pp65 antigen. Then antigenemia disappeared and did not recur until 27 weeks after HSCT, when investigations had been discontinued, and the patient unfortunately died of aspergillosis 32 weeks after HSCT.

Concerning occurrence of acute severe GVHD there was neither correlation with a development of CMV disease nor inverse correlation with the frequency of CMV-specific T cells.

pp65-specific T cells are predominantly terminally differentiated effector cells

In three patients with high numbers of CMV-specific T cells, responding cells were selected by the IFN- γ -secretion assay and flow cytometric analysis was performed to further characterize their immunological phenotype. One patient underwent three phenotypic investigations at different time points after allo HSCT. Apart from the expression of CD27, CD45RA and HLA-DR, that of the chemokine receptor CCR7 on CD8+/IFN- γ -producing cells was studied in order to differentiate between effector memory and naive T cells. Staining was positive for CD8 in a mean of 70% of the cells (range 40–87%), and most of these were CD45RA-positive (89%, range 84–95%). As expected, the majority of the cells were HLA-DR-positive as a sign of activation (86%, range 74–100%). Only 22% (range 9–33%) were CD27-positive and only 11% (range 5–21%) CCR7-positive. The pattern of these cell surface markers is compatible with a terminally differentiated effector phenotype (CD45RA+/CCR7-). Unstimulated cells as well as those stimulated with the HIV-derived peptide served as negative controls and did not produce IFN- γ .

Impact of pretransplant ATG administration on CMV-specific T-cell reconstitution

To address the question of whether ATG influences CMV-specific T-cell reconstitution, we retrospectively related ATG administration in the intermediate-risk patients to the IFN- γ ELISPOT assay response. In total, ATG was given

to 12/24 (50%) intermediate risk patients before HSCT. 8/12 (67%) patients reconstituted strong and lasting pp65-specific T-cell frequencies soon after transplantation. 7/12 (58%) patients not given ATG also reconstituted strong and lasting peptide-specific T-cell immunity soon after transplantation. Patients with and without ATG did not differ significantly in the reconstitution of a CMV-specific T-cell response.

Discussion

CMV disease is a severe complication after allogeneic transplantation and the presence of CMV-specific T cells in the graft is a prerequisite for subsequent recovery of a protective T-cell response. Reconstitution of peptide-specific T-cell immunity and rates of infection and disease are influenced by several factors, including recipient age, donor source, graft type, conditioning regimen, immunosuppressive treatment, viral load, GVHD and time to engraftment.^{1–4,6,10,15,16} Some studies demonstrate the impact of donor/recipient serostatus on higher rates of CMV infection and disease in allogeneic transplant recipients.^{3,17} The question of what donor/recipient seroconstellation might minimize the risk of CMV infection and how to best prevent CMV disease still remains controversial. There is consensus regarding the low risk of CMV infection and disease if both the donor and recipient are CMV seronegative.^{2,3,16,18} This finding is confirmed by our data, as none of our low-risk patients developed infection or disease. In an European Group for Blood and Marrow Transplantation megafile analysis, Ljungman *et al.*² studied the influence of donor CMV seropositivity on the outcome of CMV seropositive patients after unrelated HSCT. Patients allografted with CMV seropositive donors showed improved event-free survival.

In addition, various studies have demonstrated that the reappearance of CMVpp65-antigen-specific T cells after HSCT is mandatory for elimination of active infection and protection from CMV disease.^{10,12} Up to now, however, CMV-specific T-cell reconstitution has not been analyzed according to the CMV risk constellation by a functional test like the IFN- γ ELISPOT assay. The IFN- γ ELISPOT

assay has proved to be a suitable assay for monitoring pp65-specific T-cell immune reconstitution in allogeneic stem cell recipients.¹⁹ Another well-established method for assessing T-cell responses to specific peptides is the use of tetramers. Hobeika *et al.*²⁰ compared the assessment of antigen-specific T-cell reactions by peptide-MHC tetramers and IFN- γ ELISPOT assays and found the two methods to have comparable specificity and sensitivity. Despite applying two different methods for the routine CMV monitoring (PCR and antigenemia-assay) no conflicting results were obtained. As previously published, both methods are suitable for the early diagnosis of active HCMV infection²¹ and exhibit a statistically significant relationship between viral load assessed by PCR and pp65 positive leucocytes.²²

Our data suggest that a delay of CMV-specific T-cell immune reconstitution in high-risk patients causes frequent CMV infection and disease and that early recovery of T-cell immunity is linked to lower rates of CMV infection and disease. Similar observations were made by Gratama *et al.*¹⁸ who also found a protective effect and better outcome in CMV seropositive patients with CMV seropositive donors than in those with seronegative ones. Furthermore, our study revealed that, regardless of their own serostatus, recipients with CMV seropositive donors have a low incidence of CMV infection and disease. Surprisingly, the recipient serostatus had no impact on the risk of CMV infection and disease when the donor was CMV seropositive. Most of the seropositive patients allografted with a CMV seropositive donor regained a strong T-cell response within the first 100 days after HSCT. Only a minority in the d+/r- group displayed relevant T-cell responses. This finding is consistent with the results of Gratama *et al.*¹⁸ who documented the failure to retrieve CMV-specific T cells from three CMV seronegative patients allografted with cells of a CMV seropositive donor.¹⁸ The time course of specific-T cells in the d+/r- group showed decreasing T-cell frequencies, suggesting that CMV antigenemia is needed to stimulate the production of pp65-specific T cells. Intermediate-risk patients (d+/r+) usually demonstrated a strong and lasting pp65-specific T-cell response. Transient infection occurred only in two patients who never developed CMV disease. The fact that T-cell depletion increases the risk of CMV disease further confirms the importance of donor T cells with reactivity against the CMV antigens.^{19,23,24}

Taken together, CMV infection and disease rates differed significantly between high- and intermediate-risk patients, infection developing in 78 vs 12% and disease in 33% vs none. Moreover, none of the patients in the high-risk group showed recovery of specific-T cells during the phase of infection and disease, whereas up to 67% of the intermediate-risk patients displayed CMV-specific T cells.

The early increase of antigen-specific responses 4 weeks after HSCT seems to make it unlikely that naive donor T cells were activated through the antigens expressed by the CMV, as the priming of naive T cells (i.e. a primary immune response) usually requires a longer period of time. This is confirmed by the analysis of IFN- γ -producing T cells obtained from patients with elevated CMV-specific immune responses. Flow cytometric analysis revealed that most of the CMVpp65-reactive T cells were

CD8+/CD45RA+/HLA-DR+ with a weak expression of CD27/CCR7, reflecting a terminally differentiated effector phenotype.²⁵

In summary, our data demonstrated that recovery of a protective CMV-specific T-cell immune response after HSCT varies among the different CMV-risk groups and that the donor/recipient CMV serostatus constellation is of critical importance for the development of antiviral immunity and thus also for the incidence of CMV infection and disease. Therefore, CMV seropositive donors should be preferred for a CMV seropositive patient. The high risk of CMV infection and disease with a matched unrelated CMV seronegative donor is evident from the significantly higher incidence of infection and disease observed in the d-/r+ group. If a high-risk constellation cannot be avoided, peptide-specific T-cell immunity should be closely monitored to identify patients for antiviral therapy. Several studies have shown that prophylactic and preemptive antiviral therapy of those patients can decrease the incidence of viral complications like CMV after transplantation and improve survival.^{2,4,6,26,27}

Our study was restricted to HLA-A2+ patients. We therefore used the HLA A2-restricted peptide pp65, which is known to be immunogenic and immunodominant in HLA-A2+ patients.¹³ Kern *et al.* recently described a number of further immunogenic epitopes by using a pool of overlapping peptides that also allowed the inclusion of non-HLA-A2+ patients. Although the dominant response to pp65 was exclusively related to the development of CMV disease, high IE-1-specific T-cell responses correlated with protection from CMV disease.²⁸ We adapted our method and started to enroll the CMV peptide mix pool in our IFN- γ ELISPOT assay monitoring, as we consider IE-1 to be a relevant peptide. However, the available data is still too limited to draw conclusions or make comparisons. Further investigations on this approach are in progress.

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