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Brief communication

# CMV seronegative donors: Effect on clinical severity of CMV infection and reconstitution of CMV-specific immunity

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ABSTRACT

*Background:* Cytomegalovirus (CMV)-specific T-cells are crucial to prevent CMV disease. CMV seropositive recipients transplanted with stem cells from a CMV seronegative allogeneic donor ($R^+D^-$) may be at risk for CMV disease due to absence of donor CMV-specific memory T-cells in the graft.

*Methods:* We analyzed the duration of CMV reactivations and the incidence of CMV disease in $R^+D^-$ and $R^+D^+$ patients after alemtuzumab-based T-cell depleted allogeneic stem cell transplantation (TCD alloSCT). To determine the presence of donor-derived primary CMV-specific T-cell responses we analyzed the origin of CMV-specific T-cells in $R^+D^-$ patients.

*Results:* The duration of CMV reactivations (54 versus 38 days, respectively, $p = 0.048$) and the incidence of CMV disease (0.14 versus 0.02, $p = 0.003$ at 1 year after alloSCT) were higher in $R^+D^-$ patients compared to $R^+D^+$ patients. In $R^+D^-$ patients, CMV-specific $CD4^+$ and $CD8^+$ T-cells were mainly of recipient origin. However, in 53% of $R^+D^-$ patients donor-derived CMV-specific T-cells were detected within the first year.

*Conclusions:* In $R^+D^-$ patients, immunity against CMV was predominantly mediated by recipient T-cells. Nevertheless, donor CMV serostatus significantly influenced the clinical severity of CMV reactivations indicating the role of CMV-specific memory T-cells transferred with the graft, despite the ultimate formation of primary donor-derived CMV-specific T-cell responses in $R^+D^-$ patients.

## 1. Introduction

The presence of anti-viral T-cell immunity is crucial for effective and sustained protection against Cytomegalovirus (CMV) following allogeneic stem cell transplantation (alloSCT) [1]. In vitro and in vivo T-cell depletion (TCD) via addition of the anti-CD52 monoclonal antibody alemtuzumab to the stem cell graft (alemtuzumab "in the bag") is used to reduce the incidence of acute Graft versus Host Disease (GVHD) following alloSCT [2–4]. Alemtuzumab does not exclusively eliminate alloreactive T-cells, but affects presumably all T-cells, including donor-derived CMV-specific T-cells in the graft and residual CMV-specific T-cells of the recipient. Despite the profound TCD, protection against CMV is observed early after TCD alloSCT in CMV seropositive recipients ($R^+$) transplanted with a CMV seropositive donor ($R^+D^+$) mediated by CMV-specific T-cells that can either originate from the donor via transfer with the graft or from the recipient as residual memory T-cells. In CMV seropositive recipients ($R^+$) transplanted with a CMV seronegative donor ($R^+D^-$) donor-derived CMV-specific memory T-cells are not present in the graft and $R^+D^-$ patients must therefore rely on residual CMV-specific T-cells of recipient origin and/or a donor-derived primary CMV-specific T-cell response to control CMV reactivations. If despite the in vivo T-cell depletion mediated by the free alemtuzumab transferred with the graft, recipient-derived T-cell immunity predominates in the protection against CMV, the incidence and severity of CMV reactivation and disease would not differ between $R^+D^+$ and $R^+D^-$ patients. Because the function of the thymus is likely to be impaired after TCD alloSCT [5], it is not known if or when to expect a donor-derived primary immune response after TCD alloSCT. Demonstrating donor derived CMV-specific T-cells after transplantation with a CMV seronegative donor ($R^+D^-$) would be indicative of a newly developed CMV-specific primary T-cell response.

In this study we analyzed the effect of donor CMV serostatus on the incidence of CMV reactivation and CMV disease in $R^+D^-$ patients versus $R^+D^+$ patients following TCD alloSCT using alemtuzumab in the bag (20 mg). Furthermore we analyzed the origin of circulating CMV-specific $CD4^+$ and $CD8^+$ T-cell populations in $R^+D^-$ patients by

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chimerism analysis to detect donor derived CMV-specific T-cells indicative of a donor derived primary CMV-specific T-cell response.

## 2. Objectives or hypothesis

The objectives of this studies were to analyze the effect of donor CMV serostatus on the incidence of CMV reactivation and CMV disease following T-cell depleted allogeneic stem cell transplantation and to detect CMV-specific primary T-cell responses by demonstrating donor derived CMV-specific $CD4^+$ and $CD8^+$ T-cell populations in seropositive recipients transplanted with stem cells from a CMV seronegative allogeneic donor.

## 3. Material and methods

### 3.1. Patients and CMV monitoring

General institutional policy with respect to patients' informed consent for inclusion into the study, approved by the ethical institutional board, was applied. Consecutive patients transplanted in the period 2004–2010 were included. Patients with haplo-identical or cord blood transplantation were excluded from the analysis. We retrospectively analyzed CMV PCR loads, determined as part of regular post transplantation monitoring. The real-time quantitative PCR for detection of CMV DNA in plasma was performed according to the method described previously [6]. CMV DNA load guided pre-emptive therapy was initiated according to a protocol based on criteria established in a previous study [7]. CMV reactivation was defined as previously described by the detection of two consecutive positive CMV DNA loads (> log10 2.7 (> 500)/ml copies plasma) and CMV disease was defined as previously published [8]. Post transplantation sampling for T-cell analysis was scheduled every 3 months and continued for 1 year after alloSCT or longer if deemed necessary.

### 3.2. T-cell depletion and transplantation

T-cell depletion of the graft was performed by in vitro incubation of the graft with alemtuzumab (20 mg). The stem cell product was infused on day 0. Pre-transplantation conditioning was performed either according to a myeloablative (MA) conditioning protocol or a non-myeloablative (NMA) conditioning (RIC) protocol as described previously [9,10].

### 3.3. Detection and isolation of CMV-specific $CD4^+$ and $CD8^+$ T-cells based on CD137 expression

CMV-specific $CD4^+$ or $CD8^+$ T-cells were detected by flow cytometric analysis of expression of the activation marker CD137 upon stimulation of PBMC with protein spanning overlapping peptide pools of the CMV-derived proteins pp65 and IE1 [11–13]. A cluster of ≥5 $CD137^+$ events on FACS analysis within a total of 10.000 acquired events was considered positive based on the low level of background seen in CMV seronegative individuals. The isolation of CMV-specific $CD4^+$ or $CD8^+$ $CD137^+$ T-cells was performed as described previously [13]. In short, after thawing, PBMCs at a concentration of 10*10 [6]/ml were stimulated with $10^{-6}$ M CMV-derived pp65 and IE1 protein spanning peptide pools in culture medium supplemented with 10 IU/mL IL-2 (Chiron, Amsterdam, The Netherlands) for 24 h at 37 °C and 5% $CO_2$. Viability after thawing was consistently > 75%. After stimulation the cells were stained with CD137-allophycocyanin (APC, BD Pharmingen, Franklin Lakes, USA), fluorescein isothiocyanate-labelled CD16 (BD, Franklin Lakes, USA), CD14 (BD Pharmingen), CD19 (BD) and TCRγδ (BD) (dump gate), phycoerythrin (PE labelled CD4, BD Pharmingen), Alexa fluor 700 labelled CD8 (Invitrogen, Waltham, MA, USA) and PE Texas Red labelled CD3 (Invitrogen) for 30 min at 4 °C. Isolation was performed by Fluorescence Activated Cell Sorting using the FACS Aria (BD). CD16, CD14, CD19 and TCRγδ negative and CD3/CD4/CD137 triple positive and CD3/CD8/CD137 triple positive cells were sorted in bulk for chimerism analysis.

### 3.4. Chimerism analysis

Chimerism analysis on sorted CMV-specific $CD4^+$ and $CD8^+$ $CD137^+$ T-cells was performed as described previously [14]. In short, we performed PCR analysis with primers specific for patient and donor selected polymorphic short tandem repeats using the AmpFLSTR Profiler Plus ID amplification kit (Applied Biosystems, Waltham, MA, USA) and a GeneAmp 9700 thermocycler (Applied Biosystems) using AmpliTaq Gold DNA polymerase (Applied Biosystems). PCR products were analyzed using the ABI PRISM 3100 Genetic Analyzer and Genemapper V3.5 analysis software (Applied Biosystems). Maximum sensitivity of the markers was set at 2% for all markers.

### 3.5. Statistical analysis

Analysis of CMV reactivation and CMV disease was performed using competing risk analysis as described earlier [15]. Factors taken into account as competing risks were death, non-engraftment, rejection, systemic immune suppression, DLI and relapse. Additional analyses were performed using Student t-test IBM SPSS Statistics version 22.

## 4. Results

### 4.1. CMV reactivation and disease in CMV seropositive patients following TCD alloSCT

From the cohort of 157 CMV seropositive patients, 51 were transplanted with a CMV seronegative donor ($R^+D^-$) and 106 were transplanted with a CMV seropositive donor ($R^+D^+$). The donor and patient demographics (age, gender, type of conditioning regimen, unrelated/related donor) did not significantly differ between the two patient groups (Table 1). The cumulative incidences of CMV reactivations and CMV disease were compared by separate competing risks analyses, taking non-engraftment, rejection, immune suppression, DLI, relapse and death of the patient without any of these events into account as competing risks. Non-engraftment did not occur and the cumulative incidence of rejection was very low in both groups (cumulative incidence 0.02 and 0.03 in R + D- and R + D+ respectively). The cumulative incidence of CMV reactivation did not differ between the $R^+D^-$ cohort and the $R^+D^+$ cohort (0.80 versus 0.74 at 1 year after alloSCT, respectively; Gray's test p = 0.91), nor did the moment of onset of CMV reactivation after alloSCT (27 days versus 22 days, range 4–129 vs. 4–271, respectively; p = 0.7). In the patients who developed at least one CMV reactivation, the mean number of episodes of CMV reactivation was found to be similar in both groups (1.4 versus 1.4 CMV reactivations per patient in the $R^+D^-$ (n = 44) and $R^+D^+$ (n = 84) group, respectively). However, the median duration of individual CMV reactivations was significantly longer in the $R^+D^-$ cohort compared to the $R^+D^+$ cohort (54 versus 38 days, respectively, p = 0.048). The cumulative incidence of CMV disease was significantly higher in the $R^+D^-$ cohort compared to the $R^+D^+$ cohort (0.14 versus 0.02 at 1 year after alloSCT, respectively; Gray's test p = 0.003; Table 1). The cumulative incidences of the competing events non-engraftment, rejection, immune suppression, DLI, relapse and death did not differ significantly between the two groups.

### 4.2. Origin of CMV-specific T-cells in R + D- patients following TCD alloSCT

Chimerism analysis of circulating CMV-specific T-cells in $R^+D^+$ patients demonstrated recipient and donor origin, ranging from mixed donor/recipient chimerism to full donor chimerism or full recipient