Homeostasis and function of T cells in healthy individuals and renal transplant recipients

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RAPID T CELL REPOPULATION AFTER RABBIT ANTITHYMOCYTE GLOBULIN (rATG) TREATMENT IS DRIVEN MAINLY BY CYTOMEGALOVIRUS

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SUMMARY
Rabbit Antithymocyte Globulin (rATG) induces a long-lasting lymphocytopenia. CD4+ T cells remain depleted up to 2 years, whereas the CD8+ T cell compartment is refilled rapidly by highly differentiated CD27-CD45RA-CD57+ effector-type cells. Because the presence of these highly differentiated CD8+ T cells has been associated with cytomegalovirus (CMV) infection, we questioned to what extent restoration of CMV T cell-immunity contributes to the re-emergence of T cells following rATG-treatment. We compared T cell repopulation in six CMV-seropositive patients with CMV reactivation (reactivating CMV+) to that in three CMV+ patients without reactivation (non-reactivating CMV+), and to that in three CMV-seronegative recipients receiving a kidney from a CMV-seronegative donor (CMV-/-). All patients received rATG because of acute allograft rejection. Total CD4 and CD8 counts, frequency and phenotype of virus specific CD8+ T cells were determined. In reactivating CMV+ patients, total CD8+ T cells reappeared rapidly, whereas in non-reactivating CMV+ patients they lagged behind. In CMV-/- patients, CD8+ T cell counts had not yet reached pretransplant levels after 2 years. CMV reactivation was indeed followed by a progressive accumulation of CMV-specific CD8+ T cells. During lymphocytopenia following rATG treatment, serum interleuking (IL)-7 levels were elevated. Although this was most prominent in the CMV-seronegative patients, it did not result in an advantage in T-cell repopulation in these patients. Repopulated CD8+ T cells showed increased skewing in their Vβ repertoire in both CMV-/- and reactivating CMV-seropositive patients. We conclude that rapid T cell repopulation following rATG treatment is mainly driven by CMV.
INTRODUCTION

Polyclonal Antithymocyte Globulin (ATG) is used widely for various clinical conditions, including prevention and treatment of acute rejection after solid organ transplantation. It induces a rapid and massive T cell depletion causing a severe immunocompromised state leading to increased long-term risk of infection and cancer 1,2. Better understanding of the dynamics of T cell repopulation after ATG treatment may lead to improved risk-assessment.

T cell repopulation in lymphocytopenic conditions can be effectuated by thymopoiesis, homeostatic proliferation and antigen-driven expansion 3. Although each of them may play a role, it is unknown to what extent each separate process contributes. Previous studies analyzing T cell dynamics following treatment with lymphocyte-depleting agents have shown persistent depletion of peripheral CD4+ T cells. In contrast, a rapid repopulation of CD8+ T cells was observed, consisting mainly of highly differentiated CD62L-CD27-CD45RA+-CD57+ effector-type cells 4-7. In healthy individuals the presence of these cells in the circulation has been associated previously with cytomegalovirus (CMV) infection 8. During CMV latency, CMV-specific CD8+ T cells predominantly have a CD27-CD45RA+ effector phenotype 9-10.

In immunosuppressed renal transplant patients, CMV often reactivates and frequencies of CMV-specific CD8+ T cells are significantly higher than in healthy individuals 9. Here, we hypothesized that CMV infection might be an important driving factor for the rapid emergence of differentiated effector-type CD8+ T cells following lymphocyte-depleting therapy.

To study the impact of CMV infection on T cell repopulation, we compared T cell repopulation in nine CMV-seropositive to that in three CMV-seronegative renal transplant recipients treated with rATG because of acute allograft rejection. Our data demonstrate rapid CD8+ T cell repopulation in CMV-seropositive recipients with CMV reactivation. This reactivation occurred during the lymphocytopenic phase shortly after rATG treatment, and was followed by rapid and progressive accumulation of CMV-specific T cells, which contributed considerably to repopulation of the total CD8+ T cell pool.

MATERIALS AND METHODS

Subjects

We studied nine CMV-seropositive and three CMV-seronegative renal transplant recipients. The latter three received an organ from a CMV-seronegative donor. All patients had received rATG because of a second cellular rejection or acute humoral rejection (table 1). The first cellular rejections had been treated with methylprednisolone. The starting dose of rATG was 5mg/kg. In the next 14 days, three to five separate doses were administered, aimed at a depletion of T cells from the peripheral blood compartment. Dosages were titrated based on the total lymphocyte count after each administration (> 300x10E6/l: dose 5mg/kg; >200x10E6/l but <300x10E6/l: dose 3mg/kg; >150x10E6/l but <200 x10E6/l: dose 2mg/kg; <150x10E6/l: no
administration). Basic immunosuppressive treatment was similar for all patients and consisted of prednisolone, tacrolimus or cyclosporine, mycophenolate mofetil and induction with CD25 monoclonal antibody. None of the patients were treated with immunosuppressive drugs prior to transplantation. CMV-seropositive recipients were pre-emptively treated with valganciclovir in therapeutic dose, adjusted to renal function. CMV DNA was monitored by polymerase chain reaction (PCR) 2-weekly.

All patients gave written informed consent; the study was approved by the local medical ethics committee.

Cell isolation
Peripheral blood mononuclear cells (PBMCs) were isolated using standard density gradient centrifugation technique and subsequently cryopreserved until analysis.

Viral diagnostics
Quantitative PCR and serostatus for CMV and Epstein-Barr virus (EBV) were analysed as previously described \(^\text{11,12}\). Reactivation of viral infection was defined as positive viral PCR in a seropositive patient.

Tetramer complexes
Human leucocyte antigen (HLA)-peptide tetramer complexes were provided by M. van Ham (Sanquin, Amsterdam, the Netherlands). For CMV we used eight different tetramers, loaded with pp65- and IE-derived peptides. For EBV we used six different tetramers loaded with BMLF1-, EBNA3A- and BZLF1-derived peptides (Supporting information, Table S1).

Immunofluorescent staining and flowcytometry
A total of 500,000 PBMCs were incubated with allophycocyanin (APC)-labeled tetrameric complexes for 30 minutes at 4°C, protected from light. Monoclonal antibodies were added for 30 minutes. The following antibodies were used: CD45RA-phycoerythrin-cyanin7 (PECy7), CD4-AlexaFluor 700, CD28-peridinin chlorophyll (PerCP) Cy5.5, CD25-APC, CD45RO-PE (BD-Pharmingen, San Diego, CA, USA), CD27-APC-eFluor780, CD3-APC, CD3-PE, CD127-PerCPCy5.5 and CD31-fluorescein isothiocyanate (FITC) (eBioscience Inc, San Diego, CA, USA).

For intracellular staining, cells were fixed with 50ul buffered formaldehyde acetone solution and permeabilized by washing with 0.1% saponine in 50mM d-glucose. Cells were then incubated with anti-Ki-67-PE (BD Pharmingen). Samples were acquired on a BD FACSCanto. Analysis was performed using FlowJo Mac.

Quantification of Interleuking (IL)-7 levels in serum
Serum was separated from peripheral blood and stored at -20°C until analysis. IL-7 levels were measured using a commercially available human IL-7 enzym-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN) according to manufacturer’s instructions.
Isolation of CD8\(^+\) T cells, CD4\(^+\) T cells and CMV-specific CD8\(^+\) T cells

For isolation of CD8\(^+\) and CD4\(^+\) T cells, we labeled PBMCs with aCD3-PECy7, aCD8-APC and aCD4-PerCPCy5.5 (BD Pharmingen). For isolation of CMV specific CD8\(^+\) T cells, we stained with APC-labeled CMV tetramer, CD3-PECy7 and CD8-PerCPCy5.5. Cells were sorted on a FACsARIA (BD Pharmingen). Purity of the sorted cells was at least 95%.

Spectratyping of TCR-V\(\beta\) repertoire

RNA isolated from sorted CMV-specific cells was subjected to template switch-anchored reverse transcriptase–polymerase chain reaction (RT-PCR) by Smarter Pico PCR cDNA Synthesis Kit (Takara Bio Inc, Otsu, Shiga, Japan). T cell receptor (TCR)-V\(\beta\) PCR was performed on amplicons as described previously\(^\text{13}\). Next, samples were mixed with Genescan-500 ROX size standards and run on an ABI-3100 capillary sequencer (Applied Biosystems, Warrington, UK) in Genescan mode.

Statistical analysis

Statistical analysis was performed by a linear regression model with 95% confidence interval, using Prism 5.0 (GraphPad Software).

RESULTS

In the majority of rATG-treated patients, CMV reactivation occurs shortly after treatment

We hypothesized that CMV might be a driving factor for the rapid emergence of effector-type CD8\(^+\) T cells following rATG treatment. Here, we analyzed rATG treated patients longitudinally for the presence of CMV- and EBV-DNA by quantitative PCR. In six of nine CMV-seropositive patients we detected CMV-reactivation, and in four of eight EBV-seropositive patients an EBV-reactivation. CMV- and EBV-reactivations occurred at a median of, respectively, 12.5-14.5 days after administration of the last dose of rATG. All patients with CMV-reactivation were pre-emptively treated with valganciclovir as soon as CMV-DNA became detectable in the peripheral blood. None of these patients suffered from CMV- or EBV-disease.

After rATG-treatment, effector CD8\(^+\) T cells repopulate rapidly in CMV-seropositive patients, but far less rapidly in CMV-seronegative patients

We studied T cell repopulation by analyzing both absolute numbers of CD4\(^+\) and CD8\(^+\) T cells and CD27\(^+\)CD45RA\(^-\) naïve, CD27\(^+\)CD45RA\(^-\) memory and CD27\(^-\)CD45RA\(^+\)/- effector CD4\(^+\) and CD8\(^+\) T cells\(^\text{10,14}\). We compared repopulation of six CMV-seropositive patients who developed CMV reactivation (reactivating CMV+) to three CMV-seropositive patients who did not develop CMV reactivation (non-reactivating CMV+) and to three CMV-seronegative patients who received a kidney from a CMV-seronegative donor (CMV-/-). Patient characteristics are described in table 1. Apart from a somewhat large difference between patients in time between transplantation and rATG treatment, no other differences were observed between the three groups.
Table 1. Clinical characteristics of renal transplant recipients treated with rATG because of acute rejection

<table>
<thead>
<tr>
<th>ID</th>
<th>Rejection type</th>
<th>Rejection treatment</th>
<th>Days post-TX to rATG treatment</th>
<th>Age in years</th>
<th>Gender</th>
<th>R/D CMV serology</th>
<th>R EBV serology</th>
<th>CMV / EBV reactivation</th>
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<tbody>
<tr>
<td>CMV – seropositive patients with CMV reactivation (n=6)</td>
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<td>P1</td>
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<td>53</td>
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<td>+</td>
<td>Yes / Yes</td>
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<td>P2</td>
<td>AHR type II</td>
<td>rATG PE</td>
<td>25</td>
<td>57</td>
<td>F</td>
<td>R+/D-</td>
<td>+</td>
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<td>P3</td>
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<td>rATG</td>
<td>19</td>
<td>55</td>
<td>F</td>
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<td>rATG PE</td>
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<td>52</td>
<td>M</td>
<td>R+/D+</td>
<td>+</td>
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<td>35</td>
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CMV – seropositive patients in whom CMV reactivation was not detected (n=3)

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<tr>
<th>ID</th>
<th>Rejection type</th>
<th>Rejection treatment</th>
<th>Days post-TX to rATG treatment</th>
<th>Age in years</th>
<th>Gender</th>
<th>R/D CMV serology</th>
<th>R EBV serology</th>
<th>CMV / EBV reactivation</th>
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<td>ACR type I</td>
<td>rATG</td>
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<td>38</td>
<td>M</td>
<td>R+/D+</td>
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<td>17</td>
<td>45</td>
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<td>R+/D+</td>
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CMV – seronegative patients (n=3)

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<th>ID</th>
<th>Rejection type</th>
<th>Rejection treatment</th>
<th>Days post-TX to rATG treatment</th>
<th>Age in years</th>
<th>Gender</th>
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<th>CMV / EBV reactivation</th>
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<td>52</td>
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<td>-</td>
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<td>ACR type I</td>
<td>rATG</td>
<td>50</td>
<td>57</td>
<td>M</td>
<td>R+/D-</td>
<td>+</td>
<td>n/a / No</td>
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CMV: Cytomegalovirus, EBV: Epstein Barr virus, rATG: rabbit Anti thymocyte globulin, TX: transplantation, ACR: acute cellular rejection, AHR: acute humoral rejection, D: donor, R: recipient, PE: plasma exchange, NT: not tested, n/a: not applicable
In the reactivating CMV+ patients CD8+ T cells repopulated shortly after rATG treatment (20-50 days), whereas in the non-reactivating CMV+ patients the CD8+ T cell repopulation started later (200-400 days). In contrast, in the CMV-/- patients the CD8+ T cell number had not yet reached pre-transplant levels at 2 years after treatment (figure 1A). In both CMV+ groups, repopulating CD8+ T cells consisted mainly of CD27- effector-type and CD27+CD45RA- memory phenotype cells (figure 1B). CD27+CD8+ T cells are a subset of CD28+CD8+ T cells, which are a step further in differentiation. CD28+CD8+ T cells repopulation followed the same dynamics as the CD27+CD8+ T cells (data not shown).

CD27- and CD28- CD4+ T cells were almost absent in CMV-/- recipients (figure 1B&1C). Whereas in the CMV+ recipients, CD27- and CD28- effector-type CD4+ T cells contributed to the repopulation, this did not result in a significant difference in total CD4+ T cell repopulation between CMV+ and CMV-/- recipients.

CD27+CD45RA+ naïve CD4+ and CD8+ T cells were depleted almost completely, and repopulated very slowly (figure 1B). CD31 is a marker to identify recent thymic emigrants within the CD27+CD45RA+ naïve CD4+ T cell compartment. The CD31+ naïve CD4+ T cells showed similar dynamics in repopulation to the total naïve CD4+ T cells (figure 1D). No differences were detected when comparing the repopulation of CD27+CD45RA+ naïve CD4+ and CD8+ T cells of CMV+ to CMV-/- recipients (figure 1B).

Two of nine CMV+ patients received a kidney from a CMV-seronegative donor (R+D-) and seven from a CMV+ donor (R+D+); no differences in repopulation were observed.

In conclusion, we observed rapid repopulation and accumulation of CD8+ T cells only in the CMV+ patients and especially in the reactivating CMV+ patients, suggesting that CMV is a major driving force for CD8+ T cell repopulation following rATG-treatment.

**CMV-specific CD8+ T cells accumulate after rATG-treatment**

To study the effect of CMV on CD8+ T cell repopulation more closely, we analyzed the repopulation of CMV-specific CD8+ T cells after rATG-treatment. We used major histocompatibility complex (MHC) class I tetramers loaded with different peptides of two immunodominant CMV epitopes. CMV-specific cells were already detectable before ATG treatment, shortly (20-50 days) after rATG treatment and in time larger amounts eventually accumulated further. In the non-reactivating CMV+ patient (P7) the amount of CMV-specific cells detectable shortly after treatment was lower (figure 2A&B). To study whether the accumulation of virus-specific T cells is unique for CMV infection, we analyzed repopulation of EBV-specific T cells. Although EBV-specific CD8+ T cells became detectable shortly after rATG-treatment, they did not accumulate progressively (figure 2C).

The CMV-specific cells, emerging shortly after rATG-treatment, largely had a memory CD27+CD45RA- phenotype (figure 2D). This can explain the rapid repopulation of the total memory CD8+ T cell pool seen at 20-50 days after rATG-treatment in reactivating CMV+ patients (figure 1B). In time, the majority of CMV-specific cells differentiated to effector-type CD27- cells (figure 2D).

Thus, in response to viral reactivation, CMV-specific CD8+ T cells re-emerge quickly and eventually accumulate, contributing considerably to the rapid total CD8+ T cell repopulation.
Figure 1) Changes in T cell subpopulations after rATG treatment. (A) Longitudinal and comparative analysis of the absolute amount of T cells (CD3+) and the absolute amount of CD4+ and CD8+ T cells. (B) Longitudinal and comparative analysis of naïve (CD27+CD45RA+), memory (CD27+CD45RA-), effector (CD27-) CD4+ and CD8+ T cells, (C) CD4+CD28- T cells, (D) CD31+ naïve (CD27+CD45RA+) CD4+ T cells. Analysis was performed before ATG treatment (preATG), 20-50, 100-200, 200-400 and 400-700 days after ATG treatment. Black dots (n = 5 at 400-700 days after rATG treatment, n = 4 at all other time-points) represent the reactivating CMV+ patients. The grey squares represent the non-reactivating CMV+ patients (n = 3 at 100-200 days and 200-400 days after rATG treatment, n = 2 at all other time points). The open triangles represent the CMV-seronegative patients (n = 3 at preATG and 400-700 days after rATG treatment, n = 2 at all other time points). Mean absolute numbers are shown, the standard deviation (s.d.) is shown when n > 3.
Figure 2) Repopulation of cytomegalovirus (CMV)- and Epstein-Barr virus (EBV)-specific CD8⁺ T cells following rATG treatment. (A) Longitudinal analysis of CMV-specific cells, demonstrated by tetramer staining for three reactivating CMV⁺ patients (P1, P3 and P5) and one non-reactivating CMV-seropositive patient (P7) using 4 different CMV tetramers (IE 1 B8 QIK, pp65 B7 TPR, pp65 A2 NLV, pp65 B35 IPS.). (B) Longitudinal analysis of CMV - and (C) EBV specific cells. Analysis was performed before ATG treatment (preATG), 20-50, 100-200, 200-400 and 400-700 after ATG treatment. Mean and standard deviation (s.d.) of absolute numbers of tetramer positive cells are shown. To study CMV-specific cells, a total of six patients were analyzed using eight different CMV tetramers. The black bars represent five reactivating CMV⁺ patients (P1 to 5) and the gray bar represents one non-reactivating CMV⁺ patient (P7) analysed with three different tetramers. To study EBV-specific cells, five patients (four CMV⁺ patients and one CMV-seronegative patient) were analyzed using six different EBV tetramers. The black bars represent four reactivating EBV-seropositive patients and the gray bars one non-reactivating EBV-seropositive patient. (D) Longitudinal analysis of CMV-specific, tetramer positive CD8⁺ T cells. Black bars are the effector (CD27⁻) cells and gray bars the memory (CD27⁺CD45RA⁻) cells as a percentage of the CMV specific CD8⁺ T cells. n.d. = not determined
Shortly after rATG-treatment serum IL-7 levels are increased and show a positive correlation with the amount of dividing peripheral blood T cells. Next, we questioned whether serum IL-7 levels influence the repopulation of the peripheral T-cell compartment and whether CMV infection interferes with this. IL-7 is an important cytokine for T cell homeostasis and thymopoiesis. Regulation of IL-7 production is poorly understood. Various studies show elevated serum IL-7 levels during lymphocytopenia 18;19. Serum IL-7 levels are probably determined by the equilibrium between production and consumption. We observed elevated serum IL-7 levels during lymphocytopenia following rATG-treatment. Serum IL-7 levels of CMV-/ patients appeared higher than those of reactivating CMV+ patients (figure 3A).

By staining for Ki-67, we studied the number of dividing cells. Twenty to 50 days after rATG-treatment a considerable fraction of naïve, memory and effector CD4+ and CD8+ T cells were Ki-67 positive (Figure 3B&3C). We observed no differences in Ki-67 expression between reactivating CMV+ and CMV-/ patients. The fraction of dividing Ki67+ cells was largest when lymphocytopenia was most pronounced, and at the same moment there was also more free IL-7 present. The percentage of proliferating T cells positively correlated with the level of IL-7 in serum (Figure 3D, $R^2 = 0.4125$, p<0.0001).

After ATG-treatment skewing of the CD8+ TCR-Vβ repertoire increases

Given that CMV is a major driving force of T cell repopulation during rATG induced lymphocytopenia, we studied TCR-Vβ repertoire diversity of CD4+ and CD8+ T cells of two reactivating CMV+ (P1&P6) and two CMV-/ (P10&P11) patients.

The Vβ repertoire of CD4+ T cells showed a Gaussian distribution in gene scan analysis (Figure 4A&4B), before and after rATG-treatment, indicating that the repertoire remained mainly polyclonal. We did not observe differences between reactivating CMV+ and CMV-/ patients. The Vβ repertoire of CD8+ T cells generally showed a less Gaussian distribution in gene scan analysis (Figure 4C&4D). After rATG-treatment, further skewing of the CD8+ T cell repertoire was observed (Figure 4C&4D: black arrows). We quantified this skewing by calculating the difference in contribution of the area of each separate peak to the total area of each Vβ curve (Figure 4E&4F). Within the CD4+ T cells, we observed little skewing comparing the different Vβ families before rATG-treatment (preATG) to 1 year after rATG-treatment (postATG) (Figure 4E). The CD8+ T cells showed significant skewing comparing preATG to postATG (Figure 4F). No large differences in skewing were observed between reactivating CMV+ and CMV-/ patients.

Additionally, we analyzed the TCR-Vβ repertoire of 2 different CMV epitopes (pp65 A2 NLV & IE 1 A2 VLE) in 1 patient. Vβ repertoire usage of the sorted tetramer positive cells was confined to a limited number of different Vβ families. We did not observe large changes in repertoire skewing within these Vβ families comparing preATG and postATG (data not shown).
Figure 3) Changes in serum interleukin (IL)-7 levels and percentages of dividing Ki67+ T cells after rabbit anti-thymocyte globulin (rATG) treatment. Longitudinal analysis of (A) serum IL-7 levels of reactivating CMV+ patients (black line, \( n = 5 \) preATG and \( n = 4 \) at all other time points), and CMV-/- patients (gray line). Analysis was performed before ATG treatment (preATG), 20-50, 100-200 and 200-400 days after the last dose administration of ATG. Longitudinal and comparative analysis of Ki67 expression on naive (CD27+CD45RA+), memory (CD27+CD45RA-), effector (CD27-) CD4+ and CD8+ T cells. The black lines represent the reactivating CMV+ patients \( (n = 3 \) at 20-50, 400-700 days postATG treatment, \( n = 4 \) at all other time points), the grey lines represent CMV-/- patients \( (n = 3 \) preATG, \( n = 2 \) at all other time points). Mean absolute numbers and standard deviation (SD) are shown. (D) Correlation between the percentage Ki-67+ on total CD3+ cells and the amount of IL-7 present in the serum \( (R^2 = 0.4468, P < 0.0001) \). This graph represents 5 reactivating CMV+ as well as 3 CMV-/- patients.
**DISCUSSION**

CD8⁺ T cells repopulate rapidly after lymphocyte depleting treatment, whereas CD4⁺ T cells lag behind⁴⁻⁶. The repopulating CD8⁺ T-cell pool consists mainly of highly differentiated effector-type cells. We observed fast CD8⁺ T cell repopulation only in the CMV+ and not in CMV⁻⁻ patients. This rapid repopulation was most pronounced in patients who developed CMV reactivation. Thus, CMV infection appears to be a
driving factor for T cell repopulation following rATG-treatment. Indeed, when analyzing CMV-specific CD8+ T cells, we noticed a fast re-emergence and ultimately accumulation of these cells. To identify the CMV-specific CD8+ T cells, we used tetramers loaded with peptides of the most immunodominant epitopes. The shortcoming of this technique is that it is not possible to analyze the total CMV-specific response. A similar disproportionate expansion of CMV-specific CD8+ T cells has been observed during the repopulation of T cells after stem cell transplantation. Although EBV-specific CD8+ T cells also appeared shortly after rATG-treatment, they were present in much smaller numbers and did not accumulate, thus not contributing to repopulation of the total CD8+ T cell pool. In non-reactivating CMV+ patients, repopulation of CMV-specific and total CD8+ T cell was slower than in reactivating CMV+ patients. However, at 200-400 days after rATG treatment their CD8+ T cell number was completely restored while this was not the case in CMV-/- patients. We hypothesize that in non-reactivating CMV+ patients, CMV reactivated at a later time point and we failed to detect the reactivation due to less frequent sampling. Another explanation may be that local non-systemic reactivation was sufficient to drive CD8+ T cell repopulation in these patients.

Theoretically, donor specific T cells could also repopulate quickly. However, the percentage of cells responding to donor or fully mismatched third party PBMCs in mixed lymphocyte culture did not change after rATG-treatment (n=4, data not shown). CMV-infection has been associated previously with an increased incidence of allograft rejection. Furthermore, CMV-specific CD8+ T cells have been demonstrated to cross-react in allo-responses. Therefore, it will be interesting to study a possible relationship between the occurrence and severity of cellular rejection and the number of CMV-specific CD27- effector-type CD8+ T cells, but this will require a large population of patients with as compared to without acute rejection episodes.

A recent study demonstrated that, in the absence of re-exposure, tetanus toxoid specific CD8+ T cells partly survive alemtuzumab depletion and repopulate within the first year after treatment. Thus, homeostatic proliferation also contributes to the recovery of the memory CD8+ T-cell pool. Nonetheless, the presence of antigen may support repopulation even more.

Although more ‘immune space’ and a greater amount of circulating IL-7 was available, naïve CD4+ and CD8+ T cells in CMV-/- patients did not have an advantage in repopulation. Repopulation of naïve T cells can be driven by thymopoiesis and peripheral homeostatic proliferation. Shortly after rATG-treatment, we observed a larger fraction of circulating dividing Ki-67+ naïve T cells, which were presumably homeostatically proliferating in response to a surplus of IL-7. However, the presence of homeostatically cycling peripheral naïve T cells did not lead to a marked increase in naïve T cell counts. The repopulating naïve CD4+ T cells were largely CD31+, indicating that the thymus contributes to a large extent to naïve T cell repopulation, as shown in previous studies.

Although we demonstrated that CMV reactivation is an important driving force in T cell repopulation, we did not detect a larger fraction of dividing Ki-67+ T cells within the effector CD8+ T cell compartment of reactivating CMV+ recipients. Possibly, the CMV-specific CD8+ T cells become activated in a different lymphoid compartment, for example in lymph nodes.
A previous study analyzing T cell repopulation following alemtuzumab treatment found that repopulating CD8+ T cells existed mainly of CD28-CD8+ T cells. The authors propose that CD28-CD8+ T cells compete for ‘immune space’ with CD4+ T cells. We here demonstrate that after rATG-treatment CD28 CD27-CD8+ T cells did not repopulate as rapid in the CMV-/- recipients as in the CMV-seropositive recipients. However, this did not result in a benefit for CD4+ T cell repopulation in CMV-/- patients. Several other studies imply that competition for ‘immune space’ is not that relevant in T cell homeostasis. The emergence of large numbers CMV-specific cells during primary CMV infection results in transiently decreased percentages of other virus-specific memory T cells, but the absolute numbers do not diminish. Furthermore, a murine model of repetitive antigen challenge has demonstrated that the CD8+ T cell compartment is remarkably flexible and grows in size with immunological experience. The total CD8+ T cell pool in CMV infected individuals is significantly increased in size, indicating that also the human immune space is very flexible.

Because CMV has such a great effect on repopulation of CD8+ T cells, we expected to retrieve an imprint in TCR repertoire skewing selectively in CMV reactivating seropositive patients. However, in the CMV-/- patients we observed nearly the same extent of skewing in CD8+ T cell repertoire. This may be explained by the fact that CMV+ recipients already have a more oligoclonal CD8+ T cell repertoire before ATG treatment.

Our observations imply that CMV-driven T cell repopulation is the main driving factor behind the rapid CD8+ T cell repopulation following rATG-treatment for acute rejection in renal transplant recipients. Thymopoiesis and homeostatic proliferation likewise contribute to the T cell repopulation, however to a much lesser extent. Due to the tardy repopulation of the naïve T cell pool after rATG-treatment, vaccine responses, infectious disease clearance and immunological defence against malignancies remains impaired for a long period of time.

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