Evidence for neo-generation of T cells by the thymus after non-myeloablative conditioning

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ABSTRACT

Background
Background and objective. We investigated immune recovery in 50 patients given either unmanipulated or CD8-depleted allogeneic peripheral blood stem cells after non-myeloablative conditioning.

Design and Methods
Fifty patients were randomized to receive either CD8-depleted (n=22) or non-manipulated (n=28) peripheral blood stem cells. The median patients age was 57 (range 36-69) years. The conditioning regimen consisted of 2 Gy total body irradiation with or without added fludarabine. Twenty patients received grafts from related donors, 14 from 10/10 HLA-allele matched unrelated donors, and 16 from HLA-mismatched unrelated donors. Graft-versus-host disease prophylaxis consisted of mycophenolate mofetil and cyclosporine. Immune recovery during the first year after hematopoietic cell transplantation was assessed by flow cytometry phenotyping, analyses of the diversity of the TCRBV repertoire, and quantification of signal-joint T-cell receptor excision circles (sjTREC).

Results
CD8-depletion of the graft reduced the recovery of $CD8^+$ T-cell counts in the first 6 months following transplantation ($p<0.0001$) but had no significant impact on the restoration of other T-cell subsets. Both sjTREC concentration and $CD3^+$ T-cell counts increased significantly between day 100 and 365 ($p=0.010$ and $p=0.0488$, respectively) demonstrating neo-production of T cells by the thymus. Factors associated with high sjTREC concentration 1 year after transplantation included an HLA-matched unrelated donor ($p=0.029$), a high content of T cells in the graft ($p=0.002$), and the absence of chronic graft-versus-host disease ($p<0.0001$).

Conclusions
Our data suggest that while immune recovery is mainly driven by peripheral expansion of the graft-contained mature T cells during the first months after non-myeloablative transplantation, T-cell neo-generation by the thymus plays an important role in long term immune reconstitution in transplanted patients.

Key words: hematopoietic cell transplantation, non-myeloablative, T-cell depletion, GVHD, immunity, thymus.

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The online version of this article contains a supplemental appendix.

Introduction

Allogeneic hematopoietic cell transplantation (HCT) following non-myeloablative or reduced-intensity conditioning is being increasingly used in patients with hematologic malignancies who are too old or too sick to tolerate a myeloablative allogeneic HCT,[1] and in those who have failed an autologous or allogeneic HCT following a myeloablative regimen.[2] These regimens are nearly exclusively dedicated to the treatment of tumors through immune-mediated graft-versus-tumor effects.[3] Graft-versus-host disease (GVHD) and infections are the most common complications leading to non-relapse mortality,[4,5] underlying the interest of analyzing immune reconstitution following low-intensity conditioning.[6-16]

T-cell recovery after myeloablative allogeneic HCT depends on both peripheral expansion of mature T cells contained in the graft (thymus-independent pathway), and T-cell neo-generation from donor hematopoietic stem cells (thymus-dependent pathway).[17-19] However, adequate broadening of the T-cell repertoire is ensured primarily by the latter.[20] Following myeloablative allogeneic HCT, T-cell neo-generation is considered as minimal in the early months post-HCT, most circulating T cells being the progeny of T cells infused with the graft during the first 6 months following HCT.[21] In contrast, thymic production is thought to play an important role in immune reconstitution beyond day 100 in younger patients able to tolerate myeloablative conditioning, as demonstrated by the quantification of signal joint T-cell receptor excision circles (sjTREC).[20,22-25] However, following myeloablative HCT, naive T-cell counts remain low for prolonged periods, as a consequence of the reduced survival capacity of these cells.[26]

Previous studies analyzing immune recovery in (younger) myeloablative recipients compared with (older) non-myeloablative recipients have suggested that, early after HCT, patients undergoing non-myeloablative conditioning had higher naive T-cell counts than those given a myeloablative regimen.[9] This was attributed to the persistence of host-derived naive T cells after non-myeloablative conditioning.[9,27] However, 1 year after HCT, naive T-cell counts were lower in non-myeloablative than in myeloablative recipients, suggesting that neo-generation of T cells might be low in older recipients given non-myeloablative conditioning.[9] This could be due to age-related thymic atrophy.[28] Indeed, after autologous HCT a significant thymic rebound has been demonstrated in approximately 80% of patients younger than 40 years old, in 50% of those 40 to 50 years old, but in less than 15% of patients ≥50 years old.[29] These observations raise the question of whether successful immune recovery through the thymic pathway can occur in older patients given non-myeloablative conditioning, or whether immune recovery in these patients can only occur through peripheral expansion of transplanted T cells.

CD8-depletion of the graft or of a donor lymphocyte infusion has been proposed as a way to decrease the incidence of GVHD without affecting graft-versus-tumor effects.[30-35] We recently performed a comparative study of the impact of CD8-depletion of peripheral blood stem cells (PBSC) on clinical outcomes in a group of 50 patients randomly assigned to receive unmanipulated or CD8-depleted PBSC following non-myeloablative conditioning.[36] The results of this study are summarized in Table 1. Briefly, CD8-depletion of PBSC failed to reduce the incidence of grade II-IV acute GVHD, but was associated with an increased risk of graft rejection. Relapse/progression rate and overall survival were similar in the two arms of the study.[36]

Here we analyze, on the same groups of patients given allogeneic PBSC after non-myeloablative conditioning, the importance of both peripheral T-cell expansion and thymic production on immune recovery.

Table 1. Patients.

	Unmanipulated PBSC (n=28)	*CD8-depleted PBSC (n=22)*	*p-value*
Recipient age median (range), years	57 (41-65)	57 (36-69)	NS
Donor age median (range), years	40 (18-70)	45 (27-67)	NS
Donor type, no.			
HLA-identical sibling	11	8	NS
One antigen mismatched related donor	1	0	NS
10/10 HLA-allele matched unrelated donor	8	6	NS
HLA-mismatched unrelated donor	8	8	NS
Diagnoses, no. of patients			
Acute myeloid leukemia	3	0	
Myelodysplastic syndrome/ myeloproliferative disorder	7	9	
Chronic myeloid leukemia	0	2	
Lymphoma	8	6	
Chronic lymphocytic leukemia	1	4	
Multiple myeloma	7	1	
Renal cell carcinoma	2	0	
Conditioning regimen, no. of patients			
2 Gy TBI	7	5	
2 Gy TBI + fludarabine (90 mg/m^2)	21	17	
Median (range) no. of cells infused (x 10^6/kg)			
CD34$^+$ cells	4.2 (0.8-20.2)	3.7 (0.7-12.2)	0.21
CD3$^+$ T cells	319 (80-631)	110 (56-239)	<0.0001
CD4$^+$ T cells	180 (38-406)	94 (57-220)	0.0003
CD8$^+$ T cells	130 (43-272)	4.2 (0.4-33.7)	<0.0001
Acute GVHD, no. of patients			
Grade II	7	7	NS
Grade III	1	1	NS
rade IV	4	0	0.06
Chronic GVHD, no. of patients	9	4	NS
Graft rejection, no. of patients	0	7	0.001
2-year overall survival (%)	59	53	NS

TBI, total body irradiation; GVHD, graft-versus-host disease

Design and Methods

T-cell receptor excision circles assay

sjTRECs were quantified in each sample by nested real-time polymerase chain reaction (PCR), as previously described.[26;43;44] Briefly, thawed peripheral blood mononuclear cells (PBMC) were lysed for 30 min at 56°C with

Tween-20 (0.05%), NP-40 (0.05%) and proteinase K (100 µg/mL). Cell lysis was stopped by incubation for 15 min at 99°C. Multiplex PCR amplification was achieved for sjTREC together with the CD3γ chain, used as a housekeeping gene, with specific 3'/5' outer primers for each amplicon. Cycle conditions and primers/probes sequences have been reported elsewhere.[26,43] PCR products were diluted 10-fold prior to PCR quantification using Lightcycler™ technology. The quantitative PCR conditions were: 1 min initial denaturation at 95°C followed by 40 cycles of amplification (1 second at 95°C, 10 seconds at 60°C, 15 seconds at 72°C). Fluorescence emissions were assessed after the hybridization steps. Each PCR product was run for both sjTREC and CD3γ chains in two separate Lightcycler experiments. Every sample was run in triplicate, in three different experiments. The results were first calculated as absolute number of sjTREC per 10^5 PBMC; because each PBMC contains two CD3γ chain copies, sjTREC/10^5 PBMC = (sjTREC/CD3γ) x 2×10^5. Because sjTREC are only present in lymphocytes, and because the composition of PBMC is variable, the concentration of sjTREC in peripheral blood was computed using the formula: [(sjTREC / 10^5 PBMC × PBMC/µL) /100 where PBMC/µL = (white blood cells /µL × (%lymphocytes + %monocytes)]/100. Similar adjustments for calculating absolute T-cell subset counts from their frequencies in PBMC have been made previously by other groups of investigators.[45] The nested character of this quantitative PCR is endowed with a high sensitivity (detection of one copy of sjTREC per PCR reaction).

Statistical analyses

The two-way analysis of variance (ANOVA) test with graft manipulation and time as variables was used to compare T-cell subset recovery and evolution of T-cell chimerism in recipients of unmanipulated graft and those receiving CD8-depleted PBSC. Logarithmic transformation of the data was used before ANOVA analyses. The Mann-Whitney test was used to compare graft composition, TCRBV repertoire diversity and sjTREC levels in recipients of unmanipulated grafts and those receiving CD8-depleted PBSC. Wilcoxon's matched pair test was used to compare TCRBV repertoire diversity, sjTREC concentration and $CD3^+$ T-cell counts at day 40 or 100 after HCT with values obtained in the same patients on day 365 after HCT. Spearman's correlation test was used to analyze potential associations between graft composition and counts of T-cell subsets and sjTREC levels after HCT, as well as between donor's and patient's age and sjTREC levels after HCT. The probabilities of disease progression and severe infection (defined as sepsis, cytomegalovirus disease or invasive fungal infection) from day 100 to day 365 according to immune recovery on day 100 after HCT was assessed using the Kaplan-Meier method. Statistical analyses were carried out with Graphpad Prism (Graphpad Software, San Diego, CA, USA).

To determine factors affecting the counts of $CD3^+$ T cells, naive $CD4^+$ T cells, and $CD8^+$ T cells on days 100, 180 and 365 after HCT, as well as sjTREC concentration on days 100 and 365 after HCT, multivariate linear regression models for the different T-cell subset counts and sjTREC concentrations at each time point were fitted with the SAS Reg procedure (SAS Institute, Cary, NC, USA) using forward stepwise selection. Selected models were refitted for all patients since some patients were excluded in the stepwise selection because of missing values for variables not retained in the final model. Potential factors examined were donor type (HLA-identical sibling *vs.* 10/10 HLA-allele-matched unrelated donor *vs.* HLA-mismatched unrelated donor), recipient age, donor age, graft content of $CD3^+$ T cells ($CD8^+$ T cells for $CD8^+$ T-cell counts, and $CD34^+$ cells for sjTREC concentration), acute GVHD and chronic GVHD. Logarithmic transformation of the responses was used for all models. The threshold level of statistical significance was 0.05.

Results

T-cell chimerism

Median donor $CD3^+$ T-cell chimerism levels in unmanipulated and CD8-depleted recipients were, respectively, 71 and 51% (p=0.1), 73 and 65% (p=0.5), 75 and 70% (p=0.23), 75 and 70% (p=0.3), and 89 and 81% (p=0.4) on days 28, 42, 100, 180 and 365. When analyzed together with the two-way ANOVA test, recipients of unmanipulated PBSC had higher levels of donor T-cell chimerism than had recipients of CD8-depleted PBSC (p=0.01) (*Online supplementary Figure 1A*).

Reconstitution of peripheral lymphocyte subsets

Peripheral lymphocyte subset reconstitution was analyzed in both groups of patients (*Online supplementary Figure 1*). Following transplantation, paralleling the increase in $CD3^+$ T cells of donor origin (*Online supplementary Figure 1B*), $CD4^+$, $CD8^+$, naive $CD4^+$ ($CD4^+CD45RA^+$) and memory $CD4^+$ ($CD4^+CD45RO^+$) T cells were almost completely reconstituted over the first year of follow-up (*Online supplementary Figure 1C to G*).

CD8-depletion of PBSC did not significantly affect $CD3^+$, $CD4^+$, $CD4^+CD45RA^+$, and $CD4^+CD45RO^+$ lymphocyte recovery. In contrast, it significantly reduced the reconstitution efficiency of the $CD8^+$ compartment (p<0.0001) as well as that of the $CD56^+$ lymphocyte population (p=0.01). Furthermore, the expansion of $CD3^+$ T lymphocytes of donor origin was slowed down following CD8-depletion of PBSC (p=0.06). Interestingly, we observed a correlation between the number of $CD3^+$ T cells present in the graft and $CD3^+$ T-cell counts on days 28 (Spearman's R=0.39, p=0.02) and 100 (Spearman's R=0.39, p=0.04) after HCT, but neither at 180 nor at 365 days after HCT.

T-cell repertoire reconstitution

In order to evaluate T-cell repertoire diversity in transplanted patients, we performed a spectratype analysis in

both groups of patients at 40, 100, 180 days and 1 year post-transplant. The overall T-cell repertoire complexity was evaluated by scoring the number of peaks identified in the 24 TCRBV families as described previously[42] and comparing the results from five volunteer donors. At 40 days post-transplantation, TCRBV diversity was slightly reduced in both unmanipulated and CD8-depleted PBSC recipients, as compared to healthy control individuals (mean±SD TCRBV scores were 186±5 peaks in controls, versus 166±31 peaks in the patients (p=0.09)). Moreover, despite a subsequent increase in circulating T-cell numbers during the first year post-transplantation, TCR diversity continued to decrease in both groups of patients (mean ± SD TCRBV scores in all patients: 158±40 peaks (p=0.04 in comparison to the volunteer donors) and 151±40 peaks (p=0.02 in comparison to the volunteer donors) on days 100 and 365, respectively) (Figure 1A). Despite marked differences in the number of transplanted $CD8^+$ T cells, TCRBV diversity was similar in unmanipulated PBSC and CD8-depleted PBSC recipients at all time points (p=0.68, p=0.93, and p=0.34 at 40, 100 and 365 days post-transplant, respectively). Furthermore, at day 180 after HCT, TCRBV scores in both $CD8^+$ (134±56 *vs.* 112±73 peaks, p=0.4) and $CD4^+$ (152±65 *vs.* 157±35 peaks, p=0.8) T cells did not differ significantly between unmanipulated and CD8-depleted PBSC recipients. Finally, as shown in Figure 1B-C, graft manipulation, patient-donor relationship and occurrence of chronic GVHD did not significantly affect TCRBV scores.

The observation of a time-dependent reduction of the TCRBV diversity suggests that while the initial reconstitution phase is principally dependent on peripheral expansion of transplanted mature T cells, these cells are then depleted over the first year, leading to a reduced T-cell receptor repertoire. However, this reduction of peripheral T-cell repertoire is accompanied by an increased number of circulating T cells, suggesting another source of these cells.

Thymic function

In order to analyze the contribution of *de novo* T-cell production by the thymus to the overall T-cell reconstitution, we quantified sjTREC frequencies (sjTREC/10^5 cells) and sjTREC concentrations (sjTREC/mL) in the two groups of patients on days 100 and 365 after HCT (*Online supplementary Figure 2*). At day 100 post-HCT, sjTREC frequencies and concentrations were significantly higher in patients receiving unmanipulated PBSC than in those who received CD8-depleted cells (median sjTREC frequency = 67 [1-580] and 10 [1-120] sjTREC/10^5 cells in the unmanipulated and CD8-depleted groups, respectively (p=0.003); median sjTREC concentration = 391 [9-5614] and 149 [5-1495] sjTREC/mL, respectively (p=0.008, *Online supplementary Figure 2A*). In contrast, the two groups had similar sjTREC frequencies and concentrations at 1 year post-transplant (median sjTREC frequency = 107 [3-492] and 47 [1-167] sjTREC/10^5 cells; median sjTREC concentrations = 697 [25-2136] and 1022 [129-22927] sjTREC/mL in the untreated and CD8-depleted groups respectively). Whether or not these levels of thymic output are sufficient to provide successful long-term immune recovery with broad diverse T-cell repertoires several years after HCT is the focus of ongoing studies.

Interestingly, the sjTREC concentration was inversely correlated with age in both groups of patients at 1 year post-transplant (r=-0.64, p=0.0187 and r=-0.57; p=0.2 for

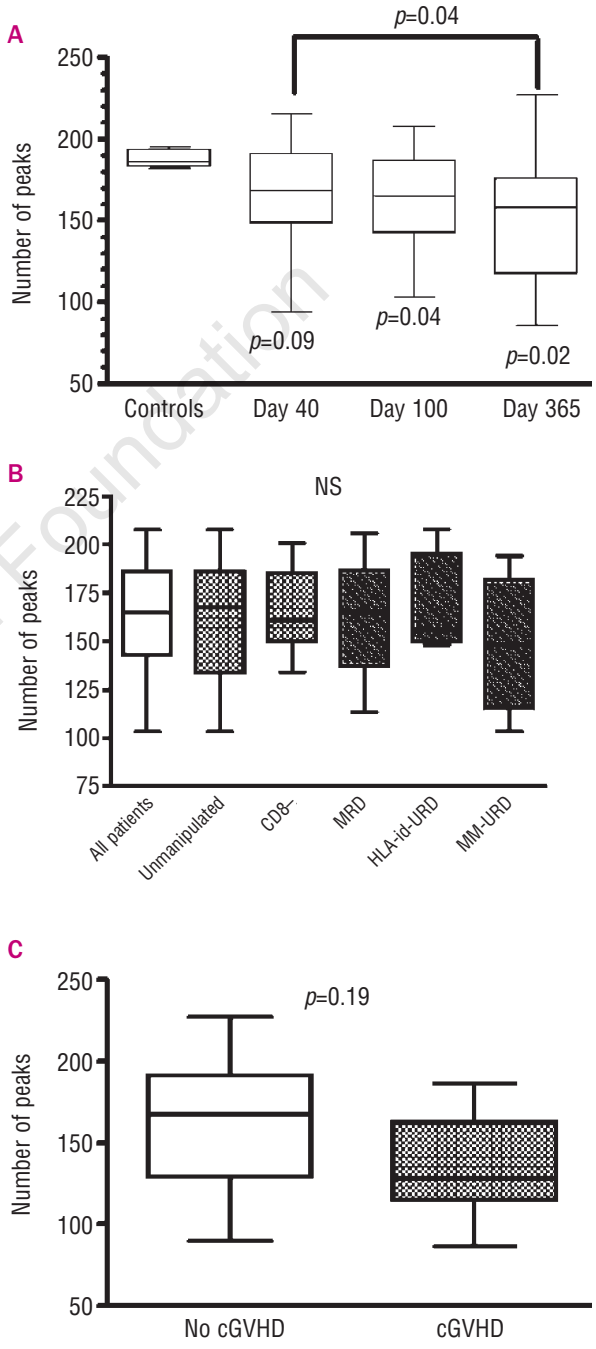

Figure 1. (A) TCRBV repertoire in five volunteer healthy blood donors, and in patients on days 40, 100 and 365 after HCT. (B) TCRBV repertoire on day 100 after HCT in patients given unmanipulated or CD8-depleted (CD8-) PBSC, as well as in patients receiving grafts from HLA-matched related (MRD), HLA-identical unrelated (HLA-id-URD), or HLA-mismatched unrelated (MM-URD) donors. (C) TCRBV repertoire on day 365 after HCT in patients with or without chronic GVHD.

the recipients of unmanipulated grafts and CD8-depleted grafts, respectively (*Online supplementary Figure 2C*); r=-0.57 and p=0.008 when all patients were analyzed together). Such a correlation, which did not exist at 100 days, suggests that *de novo* T-cell production by the thymus is an important parameter for the production of recent thymic emigrants (RTE) in transplanted patients at 1 year post-transplantation. In order to assess directly whether neogeneration of T cells through the thymic pathway occurred between days 100 and 365 after HCT, we compared sjTREC concentrations and CD3$^+$ T-cell counts on days 100 and 365 after HCT in a group of 16 patients for whom we had sjTREC data on both day 100 and day 365 after HCT. The median age of the patients in this cohort was 54 (range, 36-64) years. Both sjTREC concentrations and CD3$^+$ T-cell counts increased significantly between days 100 and 365 (p=0.010 and p=0.0488, respectively) (Figure 2A). Moreover, there was a suggestion of a negative correlation between higher patient's age and lower sjTREC increment from day 100 to 365 (Spearman's R=-0.45, p=0.078). Furthermore, at both time points, the sjTREC frequency strongly correlated with the sjTREC concentration [r=0.93, p<0.001, and r=0.93, p=0.007, respectively (*Online supplementary Figure 2D*)], demonstrating that peripheral proliferation does not have a major impact on the number of circulating T cells, at least in the compartment of recent thymic emigrants.

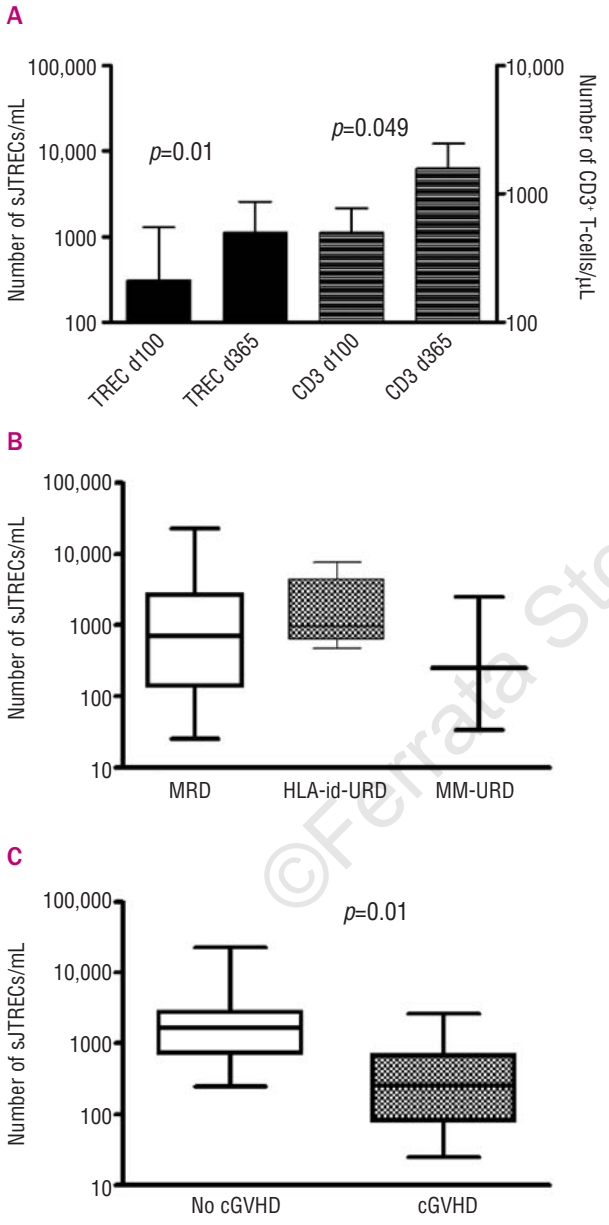

Figure 2. (A) Median sjTREC concentrations and CD3$^+$ T-cell counts on days 100 and 365 after HCT in a group of 16 patients for whom we had sjTREC data on both day 100 and day 365 after HCT. The median age of the patients in this cohort was 54 (range, 36-64) years. Error bars indicate interquartile ranges. (B) sjTREC concentrations 1 year after HCT in patients given grafts from related (MRD), HLA-identical unrelated (HLA-id-URD) or HLA-mismatched unrelated (MM-URD) donors. (C) sjTREC concentrations 1 year after HCT in patients with or without chronic GVHD.

Multivariate analysis of factors affecting immunologic recovery (Table 2)

Recipient age. High recipient age was associated with low naive CD4$^+$ T-cell counts on day 365 after HCT (p=0.02).

Donor age and type. High donor age was associated with lower counts of both CD3$^+$ T cells (p=0.02) and CD8$^+$ T cells (p=0.02) on day 365 after HCT. Having a HLA-matched unrelated donor was associated with high sjTREC concentrations on day 365 after HCT (p=0.03) (Figure 2B).

Graft composition. High numbers of transplanted CD3$^+$ T cells were associated with high CD3$^+$ T-cell counts on day 100 after HCT (p=0.01). This association was not present on day 180 or 365 after HCT. High numbers of transplanted CD3$^+$ T cells were also associated with high sjTREC concentrations after HCT (p=0.002). High numbers of transplanted CD8$^+$ T cells were associated with high CD8$^+$ T-cell counts on days 100 (p=0.001) and 180 (p=0.04) after HCT. This association was not present on day 365 after HCT. There was no association between numbers of transplanted CD34$^+$ cells and sjTREC concentrations on day 100 or 365 after HCT.

GVHD. The occurrence of grade II-IV acute GVHD was associated with lower counts of both CD3$^+$ T cells (p=0.02) and CD8$^+$ T cells (p=0.049) after HCT. The occurrence of chronic GVHD was associated with a low sjTREC concentration on day 365 after HCT (p<0.001) (Figure 2C).

Associations between immune recovery and disease progression/infections

There was no significant association between the occurrence of severe infections (defined as sepsis, cytomegalovirus disease or invasive fungal disease) on days 100 and 365 after HCT in patients with sjTREC concentrations, CD3$^+$ cell counts or CD4$^+$CD45RA$^+$ cell counts below or above the median. Similarly, there was no significant association between the incidence of disease progression on days 100 and 365 after HCT in patients with sjTREC concentrations, CD3$^+$ cell counts or CD4$^+$CD45RA$^+$ cell counts below or above the median.

Discussion

Whether or not successful immune recovery through the thymic pathway can occur in older patients given non-myeloablative conditioning has remained an important unsolved question. In this study, we evaluated immune recovery in patients receiving allogeneic PBSC transplantation after non-myeloablative conditioning. This study was part of a prospective randomized trial aimed at assessing the impact of CD8-depletion of PBSC on the risk of development of GVHD in patients who cannot undergopre-transplant myeloablative treatment. We show that the immune recovery period can be divided into two major phases. During the first 3 months post-transplantation, most of the immune reconstitution is principally a consequence of the expansion of the mature T cells present in the graft. However, *de novo* production by the thymus cannot be excluded during this period. In contrast, following this early phase, the thymus seems to be a major source of circulating T cells in the patients.

There was a strong correlation between graft composition and both $CD3^+$ T-cell counts and $CD8^+$ T-cell counts in the first 100 days after HCT, suggesting that immune recovery was mainly driven by peripheral expansion of mature T cells present in the graft early after HCT. These observations are in agreement with the results of previous studies performed in younger patients undergoing myeloablative allogeneic HCT.[46] Indeed, Hochberg *et al*. found that TREC levels remained low in the first 3 months following myeloblative allogeneic bone marrow transplantation in adults.[23] In addition, patients given T cell-depleted grafts had lower levels of both naive T cells[47] and sjTREC[24] in the first months following myeloablative conditioning, in comparison to those given unmanipulated grafts. Furthermore, naive T-cell counts 100 days after myeloablative HCT were strongly correlated with the number of naive T cells in the graft.[21] Similarly, after non-myeloablative conditioning, the number of TREC-positive cells was shown to decrease gradually in the first 6 months following HCT, suggesting that the main mechanism of T-cell reconstitution early after non-myeloablative conditioning is thymus-independent.[10] Finally, another study suggested that graft composition was the main factor affecting immune recovery during the first 6 months following non-myeloablative HCT.[7]

Importantly, our data demonstrated that neo-generation of T cells through the thymic pathway was a major source of circulating T cells 1 year after HCT. The important role of neo-generation of T cells by the thymus after non-myeloablative conditioning was rather surprising, given the fact that this strategy is mainly proposed for older patients. Indeed, in the current study, 50% of the patients were older than 57 years, and 25% older than 63. While TREC concentrations 1 year after HCT were inversely correlated with the patients' age in univariate analysis, absence of chronic GVHD, a high number of $CD3^+$ T cells in the graft and HLA-matched unrelated donor were the three main factors affecting this concentration. These findings are in agreement with previous observations in younger patients given allogeneic grafts following myeloablative conditioning, which showed that graft composition,[24] recipient age[24,48] and absence of chronic GVHD[22,24,25] were the main factors affecting thymic function the first year following HCT.[24,48] While the negative impact of chronic GVHD on thymic function could be due to both direct destruction of host thymic epithelium by chronic GVHD and the negative impact of immunosuppressive drugs,[49,50] the association between high numbers of $CD3^+$ T cells in the graft and high sjTREC concentrations could be due to both a higher sjTREC content in the graft and a lower need for peripheral T-cell expansion (and thus less dilution of sjTREC) in patients whose grafts contained more T cells. The association between high sjTREC levels and HLA-matched unrelated donors could be due to both impaired thymic function (maybe through subclinical alloreactivity of donor T cells towards recipient thymic epithelium) in patients given grafts from HLA-mismatched unrelated donors,[50] and older donors' age (and thus lower frequency of prethymic T-cell progenitors) in patients given grafts from HLA-identical siblings.[14] Although we did not perform computed tomography scans of the thoracic inlet to demonstrate newly emerging thymic shadows, previous studies found a strong correlation between thymic size increment and sjTREC levels after HCT,[29] suggesting that sjTREC-positive cells are mainly generated by the thymus also in the HCT setting.

Finally, our study showed that the T-cell repertoire remained relatively diversified early after HCT, but was more restricted 1 year after HCT. While the relatively complex T-cell repertoire early after HCT could be partly

Table 2. Multivariate analyses of factors affecting immune recovery after non-myeloablative conditioning*.

Cell subset	*Day after HCT*	*Factor(s) associated with higher cell subset counts*
$CD3^+$ T cells	100	High no. of $CD3^+$ T cells transplanted† (p=0.010)
		Absence of grade II-IV acute GVHD (p=0.021)
	365	Low donor age† (p=0.017)
Naive $CD4^+$ T cells	365	Low recipient age† (p=0.021)
$CD8^+$ T cells	100	High no. of $CD8^+$ T cells transplanted† (p=0.001)
		Absence of grade II-IV acute GVHD (p=0.049)
	180	High no. of $CD8^+$ T cells transplanted† (p=0.036)
	365	Low donor age† (p=0.015)
sjTREC	365	10/10 HLA-allele-matched unrelated donor (p=0.029)
		Absence of chronic GVHD (p<0.0001)
		High no. of $CD3^+$ T cells transplanted† (p=0.002)

**Potential factors examined were donor type (HLA-identical sibling vs. 10/10 HLA allele-identical unrelated donor vs. HLA-mismatched unrelated donor), recipient age, donor age, graft content of $CD3^+$ T cells ($CD8^+$ T cells for $CD8^+$ T-cell counts, and $CD34^+$ cells for sjTREC concentration), acute GVHD and chronic GVHD. †continuous linear variable; No., number; CMV, cytomegalovirus; TBI, total body irradiation; CSP, cyclosporine; MMF, mycophenolate mofetil; GVHD, graft-versus-host disease.*

due to the persistence of host-derived T cells,[9,27] the more restricted T-cell repertoire seen at 1 year could be the consequence of gradual eradication of residual host-derived T cells by donor-derived T cells,[9,27] or death of mature T cells contained in the graft following their massive expansion. Alternatively, since analysis of the T-cell repertoire cannot definitively distinguish between T-cell loss and T-cell expansion, the more restricted T-cell repertoire observed at 1 year could be due to massive clonal expansion of specific memory T-cell clones, such as cytomegalovirus-specific clones, or clones causing GVHD.[51]

In summary, our results suggest that while immune recovery was mainly driven by peripheral expansion of mature T cells contained in the graft during the first 3 months after non-myeloablative transplantation, T-cell neo-generation by the thymus plays an important role in long-term immune reconstitution in transplanted patients.

Authorship and Disclosures

EC performed the sTREC analyses, analyzed the data, and edited the paper; FB designed the study, analyzed the data, and wrote the paper; EW took care of the patients, and analyzed the data; NS-L and AG performed the flow cytometry analyses and edited the paper; NM performed the TCRVB analyses; J-FV and CH performed the chimerism analyses; LS performed the multivariate statistical analyses and edited the paper; VG helped with sjTREC analyses and edited the paper; RC helped with sjTREC analyses, helped with the analyses of the data and with the writing of the paper; YB designed the study, took care of the patients, analyzed the data, and edited the paper. All authors approved the final (revised) version of the manuscript. The authors reported no potential conflicts of interest.

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