Evolution of the donor T cell repertoire in recipients in the second decade after allogeneic stem cell transplantation

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**Running title:** Lymphocyte reconstitution after transplantation

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ABSTRACT

After allogeneic stem cell transplantation (SCT), T lymphocyte function is re-established from the donor’s post-thymic T cells and through thymic T cell neogenesis. The immune repertoire and its relation to that of the donor have not been characterized in detail in long term adult SCT survivors. We studied 21 healthy individuals in their second decade following a myeloablative SCT for hematological malignancy (median follow-up 12 years). Immune profiles were compared with donor samples cryopreserved at transplant and beyond 10 year from SCT. Only one recipient was on continuing immunosuppression. Compared with the donor at transplant there was no significant difference in CD4, CD8, NK, and B cell blood counts. However, compared to donors, recipients had significantly fewer naïve T cells, lower T cell receptor excision circle levels, fewer CD4 central memory cells; more effector CD8+ cells, and more regulatory T cells. T cell receptor (TCR) repertoire analysis showed no significant difference in complexity of TCRVβ spectratype between recipients and donors, although spectratype profiles had diverged with both gain and loss of donor repertoire peaks in the recipient. In conclusion, long term allogeneic SCT survivors have subtle defects in their immune profile consistent with defective thymic function but compatible with normal health. This study is registered at www.clinicaltrials.gov as NCT00106925.
INTRODUCTION

Ninety percent of allogeneic stem cell transplantation (SCT) recipients alive two years after SCT will become long-term survivors,¹ and as survivors age more attention must be paid to their long-term status and potential complications.²

After SCT prolonged immune deficiency and delayed T cell reconstitution results in significant morbidity and mortality. Full immune recovery after SCT implies a normal distribution and number of lymphocyte subsets and antibody production, and immune competence against infectious agents, immunosurveillance of malignant cells and absence of active graft-versus-host disease (GVHD). While many studies show that most immune parameters return to a normal range within a few years of SCT, recipient age and occurrence of chronic GVHD (cGVHD) govern the pace and completeness of immune reconstitution.³ In adult SCT recipients, thymic failure limits the contribution of new donor-derived naïve T cells to the repertoire, which is supplied from long-lived post thymic T cells transfused with the stem cell graft.⁴ GVHD further impairs full immune recovery, in part through an associated immune imbalance, and in part because GVHD damages the thymus. In addition, immunosuppressive treatment for GVHD impairs immune function. Despite these barriers to complete normalization of the immune system, many adult recipients become healthy long-term post-transplant survivors. However there is no comprehensive data on the immune profile of recipients surviving into their second decade following SCT. In particular it is not known how much of the immune repertoire is derived from post-thymic T cells and how much it is complemented by new thymic emigrants. Here we describe the immune characteristics of 21 healthy
individuals surviving into their second decade after myeloablative T cell depleted SCT for leukemia. Availability of cryopreserved donor samples from the time of transplant permitted paired comparisons between recipient and donor and revealed greater evolution of the donor T cell repertoire in the recipient than within the donor over the same time period.

**PATIENTS AND METHODS**

**Study Design** These data were drawn from an NHLBI/NIH Institutional Review Board approved long-term follow-up study of 121 recipients who received a T cell depleted SCT from an HLA identical sibling donor between 1993 and 2004 (NHLBI 2005-H-0130, registration number: NCT00106925). Consent was obtained in accordance with the Declaration of Helsinki. Forty-two of 121 recipients survived 10 or more years. Four of these died (two from chronic GVHD, two from cardiovascular causes). Twenty-one recipients and 15 of their donors consented to participate in this study, requiring blood sample collection for immunological studies. A cross-sectional analysis of clinical outcomes and T cell reconstitution was completed in a sample of recipients surviving into their second decade following allogeneic SCT.

**Transplantation Protocols** All recipients received cyclophosphamide 120 mg/kg and 12-13.6 Gy total body irradiation conditioning. Recipients received T cell-depleted bone marrow (BM) (n=15) or G-CSF mobilized peripheral blood (PBSC) SCT (n=6) with cyclosporine for GVHD prophylaxis and delayed add-back of donor lymphocytes 30-90
days post transplant. Acute and chronic GVHD were graded based on published criteria.⁵,⁶

**T Cell Depletion** All transplants were T cell depleted *ex vivo*. In the first protocol (93-H-0212), the bone marrow harvest was depleted of T cells by elutriation.⁷ In protocol 97-H-0099, T cells were depleted by CD34⁺ selection on the Ceprate SC column (CellPro, Bothell, Washington), followed by CD2 selection on a second column (CellPro, Bothell, Washington). In subsequent protocols, T cells were depleted from the graft using the Isolex 300i immunomagnetic cell selection system (Baxter Healthcare, Deerfield, Ill) for positive selection of CD34⁺ cells, followed by negative selection of T cells using an antibody cocktail of anti-CD2, anti-CD6, and anti-CD7, as previously described.⁸

**Donor Lymphocyte Infusion** To prevent relapse and facilitate immune reconstitution, one or two lymphocyte infusions (total dose 1-10 x 10⁶ CD3 cells/kg) were given between days +30 and +90, provided the recipient was not receiving steroid treatment for acute GVHD grade >II.

**Reagents for Flow Cytometry** The following reagents were used for immunophenotypic analyses of leukocyte subsets: i) αCD14 pacific blue, αCD19 pacific blue, αCD8-APC Alexa 750, αCD45RO-PE, and ViViD (Live/Dead Fixable Violet Dead cell staining kit) (Invitrogen, Burlingame, CA); ii) αCD56-fluorescein isothiocyanate (FITC) or PECy5.5, αCD57 FITC, αT cell receptor (TCR) γδ-phycoerythrin (PE), αCD19-PECy5, αCD3-PECy7, αCD16-APCCy7, αCD4-allophycocyanin (APC), and αFOXP3 Alexa 647 plus
the buffer set (Becton Dickinson Biosciences, San Jose, California); iii) αNKG2A-PE (CD159a), αNKG2D-APC (CD314), and αCD27-PECy5 were from Beckman Coulter (Miami, FL).

**T Cell Surface and Intracellular Staining for Flow Cytometry** Flow cytometric analysis was performed as described previously.9,10 Briefly, peripheral blood mononuclear cells (PBMC) were thawed, rested overnight at 37°C in RPMI 1640 supplemented with 10% heat-inactivated human AB serum (Gemini Bio-Product, Woodland, CA), 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA) with 50 units DNase I (Roche)/mL, and stained with ViViD (Live/Dead Fixable Violet Dead cell staining kit, Invitrogen L34955) for 15 minutes at room temperature (rT). After washing, the cells were stained with the appropriate monoclonal antibodies (mAb) for 15 minutes at rT, washed again, and acquired on a Canto II flow cytometer (Becton Dickinson Biosciences, San Jose, CA). For the staining of intracellular markers, the cells were first stained with ViViD and anti-surface antigen mAb, fixed, permeabilized, and stained with anti-FoxP3 mAb.11,12 Data were acquired on a Canto II flow cytometer. The data were analyzed using DiVa (BD Biosciences, version 5.0.1). Using this panel of mAb we defined naïve, central memory, central effector, and effector T cells as follows: Regulatory T cells (Tregs), CD4+CD25+FOXP3+; naïve T cells, CD27+CD45RO-CD57-; central memory T cells, CD27+CD45RO+CD57-; effector memory T cells, CD27-CD45RO+CD57+/-; effector T cells, CD27-CD45RO-CD57+. 
Flow Cytometric cell sorting  PBMC were thawed and rested overnight at 37°C, and stained with ViViD for 15 min at room temperature. Following a wash step with FACS buffer (PBS with 2% FCS and 0.05% sodium azide), the cells were stained with mAb to CD14, CD19, CD3, CD4, and CD8, and sorted on a Vantage flow cytometer (BD Biosciences). A minimum of 300,000 CD4+ or CD8+ T cells were sorted as dry pellets and into RNA isolation reagent Trizol (Invitrogen) for T cell receptor excision circle (TREC) and TCR spectratype analysis, respectively.

TREC Analysis  TRECs were quantified in sorted CD4+ and CD8+ T cells as described. In brief, cells were lysed in 100 μg/mL proteinase K for 1 hour at 56°C, after which the enzyme was heat-inactivated for 10 minutes at 95°C. Real-time quantitative PCR was performed on 5 μL of cell lysate with an ABI7700 system (Applied Biosystems, CA) as previously described. A standard curve was generated from pZero Blunt (pCR Blunt) plasmid (Invitrogen, Carlsbad, California) and TREC values for samples were calculated by the ABI7700 software. Samples were analyzed in duplicate.

TCR Spectratyping  Total RNA was extracted from the sorted CD4+ and CD8+ T cells, reverse-transcribed and examined for spectratypes as described. In brief, cDNA aliquots were amplified to saturation with 1 of the 23 TCRVβ oligonucleotide primers in combination with a fluorescently labeled constant region primer. Amplicons were denatured in gel loading buffer and loaded onto a denaturing polyacrylamide sequencing gel and analyzed in an automated DNA sequencer for size fluorescence intensity determination (spectratyping profile). Applied Biosystems GeneMapper software was
used for quantitative repertoire analysis of Vβ subfamilies. Overall complexity within a Vβ subfamily was determined by counting the number of discrete peaks for a Vβ subfamily, with each subfamily graded on a score of 0 to 5.16 Spectratypes containing more than 5 peaks were given a score of 5; spectratypes with 0, 1, 2, 3, or 4 peaks were given a score of 0, 1, 2, 3, or 4, respectively. The maximum complexity score for a sample is 115 (23 Vβ X 5).17 A diversity score was created to describe differences in spectratype between recipient and donor reference samples. A score of 1 was given either for peak heights ≥2 x than that of the reference sample or ≤½ the height of the reference sample. The sum of these scores for CD4 or CD8 T cells is the diversity score.

**Statistics** Descriptive statistics were used to summarize the demographic and clinical characteristics. The risk factors associated with long-term outcomes in the recipients who survived the first 10 years post-SCT were determined by analyses of clinical and transplant variable characteristics of recipients. Two-sided Fisher’s exact tests or Chi-squared tests were used in univariate analyses for categorical variables. The paired t-test and Mann–Whitney U-test were used for continuous variables. Statistical significance was accepted at p value less than 0.05. The data analyses were performed using the Prism 4 for Windows (GraphPad Software, Inc., La Jolla, CA).
RESULTS

Patient Characteristics Of 121 recipients enrolled in a long-term evaluation protocol, 21 of 42 surviving SCT a median of 12 years (range 10-15) were studied. The indication for transplant was chronic myelogenous leukemia (17), acute myelogenous leukemia and myelodysplastic syndrome (3), and multiple myeloma (1). All recipients received donor lymphocyte infusion 30-90 days post transplant. Recipient characteristics are summarized in Table 1. Median age at SCT was 36 years (range 13-56) for recipients, and 36 years (range 16-58) for donors. Previously, 6 (29%) had developed acute GVHD (grade II-IV) and 18 (86%) chronic GVHD (13 limited, 5 extensive) (Table 1). Six (29%) recipients were on prolonged immunosuppressive therapy (IST) for cGVHD, 3 for up to 5 years and 2 from 6-11 years. One recipient with extensive cGVHD remained on treatment at the time of study. At the time of study all recipients were well with no intercurrent infection or disease relapse.

Quantitative Immunoglobulins Quantitative immunoglobulins were measured in 21 recipients in their second decade after transplant. Median concentrations (+/- 95% CI) for IgG, IgA and IgM were 1030 mg/dL (952-1209); 198 mg/dL (177-264) and 106 mg/dL (101-157) respectively. All values were well within the normal ranges [IgG (642-1730); IgA (91-499) and IgM (34-342)] (Table 2).

Lymphocyte Counts Absolute CD3+, CD3+/CD4+, CD3+/CD8+ and NK (CD3-/CD56+) counts were measured in 18 recipients and were within the normal range. CD19+ counts
(median 453/uL) were elevated compared to the normal population (range 61-321/uL) (Table 2).

**Comparisons with donor at time of SCT** Leukocyte subsets were compared in donor-recipient pairs using donor cells obtained at transplant. There was no significant difference in the total lymphocyte, neutrophil, monocyte, NK cell, CD4+ and CD8+ T cell, and B cell absolute counts compared with the donor pre-transplant (Figure 1A). However SCT survivors had significant differences in CD4+ and CD8+ T cell and NK cell subset composition compared with the donor sample taken at transplant: In the CD4 compartment, recipients had significantly more Tregs (as a percentage of CD4 cells, almost double in the recipient compared with the donor, \( p = 0.02 \)) (Figure 1B), and significantly fewer naïve \( ( p = 0.003) \) (Figure 1C), and central memory cells \( ( p = 0.03) \) compared to their donors. In the CD8 compartment, recipients had more effector \( ( p = 0.02) \) and fewer naïve cells \( ( p = 0.0001) \) (Figure 1C). In the NK compartment, there were fewer CD56[int]CD16-NKG2A+NKG2D+ NK cells \( ( p = 0.02) \). Recipients with a history of cGVHD had a significant decrease in naïve CD8+ T cells \( ( p = 0.02 \) and a trend to decreased naïve CD4+ T cells \( ( p = 0.31) \), compared with those with no cGVHD. The recipient (UPN# 76) who had extensive cGVHD receiving immunosuppression, had the lowest number of naïve CD4+ and CD8+ T cells. In nine instances, donor blood samples contemporaneous with those from transplant survivors in this study were also obtained. In paired comparisons of donor samples taken at transplant and a median of 12 years later there was no significant difference of T cell phenotypes, including absolute lymphocyte
count, naïve CD4 and CD8 T cells, NK cells, T cell subsets, B cells, and Tregs lymphocytes (Figure 1D).

**TREC Analysis** To measure thymic output after SCT, the TREC levels were examined separately for sorted CD4+ and CD8+ T cells in donor-recipient pairs. Samples from 8 donors were sufficient for sorting CD4 and CD8 T cells to compare TREC levels with their individual recipients. TREC levels correlated with numbers of naïve CD4+ and CD8+ T cells, with lower TREC levels in the recipients ($r = 0.69, p = 0.01$) than that in the donors ($r = 0.65, p = 0.02$) (Figure 2A) suggesting persisting thymic deficiency. Compared with donor samples taken at SCT, TREC levels were lower in recipient CD4+ ($p = 0.05$) and CD8+ ($p = 0.01$) T cells (Figure 2B). However in three cases where comparisons could be made there were no significant differences in CD4+ and CD8+ T cell TREC levels between the contemporaneous donor samples and those taken at SCT (data not shown). However TREC levels were significantly lower in CD4+ T cells of recipients with history of GVHD than that of the cohort of recipients without history of GVHD ($p = 0.02$). The recipient (UPN# 76) with extensive cGVHD receiving immunosuppression, had the lowest TREC levels in CD4+ or CD8+ T cells.

Although there was a trend to lower TREC levels in older individuals, differences in TREC levels in either recipients or donors age $>36$ years at SCT compared with younger individuals were not significant (data not shown).
**TCR repertoire spectratyping** TCR repertoire analysis was used to evaluate thymic function. Total RNA was extracted from sorted CD4+ or CD8+ cells and spectratype profiles of the 23 Vβ subfamilies were analyzed in recipient and their donors at SCT. The samples from 6 donors had sufficient sorted CD4 and CD8 T cells for RNA extraction to compare TCR repertoire with their individual recipients. A representative TCR Vβ spectratype from one donor-recipient pair is shown in Figure 3. The recipient shows a full normal-looking repertoire in the second decade after transplant. The TCRVβ diversity (total complexity score) describes the polyclonality of the T-cell repertoire. There was no significant difference in total complexity scores of T cells between donors at SCT (CD4+, mean ± SD: 101 ± 3)(CD8+, mean ± SD: 100 ± 3) and recipients (CD4+, mean ± SD: 104± 2) (CD8+, mean ± SD: 101 ± 2) (Figure 4B). However, spectratyping profile diversity showed significant differences between recipients and donors. Figure 4A shows an example of pattern diversity between donor and patient with both gains and losses of repertoire peaks in the recipient. The median diversity score for CD4+ and CD8+ T cells was 18 ± 1.6 and 21 ± 0.8 respectively (Figure 4D). The altered but diverse TCRVβ spectratyping profiles indicate considerable TCR repertoire plasticity in the recipient. We also compared changes in the TCRVβ repertoire in 4 donors at transplant and in the second decade after SCT. Over the period between sample collections there was no significant difference in total complexity scores of CD4+ cells or CD8+ T cells (CD4+, mean ± SD: 99 ± 4.3 vs. 100 ± 4.3; CD8+, mean ± SD: 97.5 ± 3.6 and 99 ± 2.9) (Figure 4C). There was evidence of TCR repertoire plasticity with a diversity score of 10.2 ± 0.7 for both CD4+ and CD8+ T cells. However this represented less diversity than was seen between recipient and donor (Figure 4E).
TCRVβ spectratype from a donor sample at SCT and more than a decade later is shown in Figure 3.

**DISCUSSION**

It is well established that immune recovery after SCT is prolonged and incomplete and T cell immunity may remain impaired for years after SCT. Naïve T cells of donor origin appear around 6 months after SCT but restoration of the naïve T cell pool may require 1 to 2 years and may remain incomplete especially in recipients aged >45 years. Naïve T cells recovery after SCT is much slower in adults than in children reflecting an age associated post-SCT thymic rebound.

Here we describe the first detailed immune profiling of adult transplant recipients in the second decade after SCT. Although absolute values of T cells, NK cells and immunoglobulins were in the normal range, we found more subtle differences in T cell and NK cell subsets when recipients were compared with their donors. Notably, recipients still showed reduced frequencies of naïve T cells and memory CD4 T cells. Together with the reduced TREC levels, these findings point to a persisting defect in thymic output in these long-term SCT survivors. These data from adult SCT recipients contrast with the report from Cavazzana-Calvo et al. in primary T-cell-immunodeficient children who had undergone SCT over 10 years ago. All recipients had persistent thymopoiesis despite myeloablative conditioning with presence of naive T cells carrying TRECs.
Because many adult recipients have a history of GVHD, it was not possible to determine whether age alone inversely correlated with TREC levels. Although there was a trend for older recipients to have lower TREC levels, there was no significant decrease in TREC levels in the donors over the 10-year interval. Both SCT conditioning and GVHD contribute to reduced thymic function. We found that patients with a history of chronic GVHD had decreased numbers of naïve T cells and levels of TREC compared with those with no chronic GVHD, and the single recipient studied with active cGVHD had the lowest TREC levels. These data are consistent with previous reports indicating that thymopoiesis is inhibited by GVHD. An interesting observation was that recipients had significantly higher numbers of CD4+CD25+FOXP3+ regulatory T cells. The reason for this is unclear. We did not study the activity of the regulatory T cells detected, and it is unclear whether these Tregs contribute to the relative tolerance observed in these patients. It is possible that Tregs increase in response to donor alloimmune responses and remain high even after cGVHD has become quiescent. It is also possible that the regulatory cells detected caused excess turnover of mature T-cells, thereby inducing more rapid dilution of TREC in these patients. Alternatively, the thymic precursor pool may have been more limited. These Tregs would reduce TREC irrespective of the status of the thymic microenvironment. On the other hand, one study from Ritz’s group found that the regulatory T cell frequencies are reduced in patients with active cGVHD, but the function of these Tregs remains normal.
In the first year after transplantation, TCR repertoires remain abnormal and TREC values low,\textsuperscript{22} implying that the recovery of the full T cell receptor repertoire requires restoration of thymic function. Thymopoiesis after SCT has been shown to be critical for the restoration of peripheral CD4 T cell populations with broad TCR diversity,\textsuperscript{4} and de novo T-cell generation by the thymus is important in long term immune reconstitution in recipients after SCT.\textsuperscript{15} The fully diverse CD4+ and CD8+ T cell spectratypes in our survivors, in the face of a defective thymic output, raises the question how much the peripheral compartment was diversified by a slow trickle of thymic emigrants over the course of a decade and how much the peripheral T cell repertoire evolved to meet novel or persisting antigenic challenges (both allogeneic and infectious) in the recipient. Comparison of spectratypes between recipient and donor revealed a further evolution of the repertoire in the recipient - i.e. acquisition of new clonotypes and loss of other clonotypes. Consistent with the importance of the recipient allogeneic milieu in molding a new repertoire is the fact that the diversity in the recipient compared with the donor sample taken at SCT was twice as great as the change occurring within the donor over the same time period. This would be consistent with both alloantigen,\textsuperscript{23} and viral antigenic challenge from persisting viruses.\textsuperscript{24}

These observations were made in a group of healthy transplant survivors who, with one exception, were off all immunosuppressive treatment. Nevertheless although only one patient had active chronic GVHD, immune alterations related to a history of GVHD appear to persist long-term. Such relatively minor abnormalities in immune profile were compatible with good health. However, it remains to be seen whether these defects
presage worsening infectious complications when these long term survivors become elderly and experience age-related attrition of their immune function.

In conclusion, despite the evidence of poor thymic function in long-term SCT recipients the T cell repertoire appears to have evolved further in the recipients and diversified sufficiently to render these individuals immune competent.

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AUTHORSHIP

Contributions:

R.Q.L. designed the research, provided clinical care, collected data, performed experiments, analyzed data, and drafted the manuscript;

J.J.M. designed and performed experiments, analyzed data, and drafted the manuscript;

M.B. provided clinical care, collected data, analyzed data, and drafted the manuscript;

B.H. and S.M. designed and performed experiments, analyzed data and commented on the manuscript;

B.N.S. and A.S. provided clinical care, collected patient data, and commented on the manuscript;

E.K.K. provided clinical care, collected patient data and commented on the manuscript;

K.K. and N.F.H. performed experiments and commented on the manuscript;
F.T.H and D.C.D. designed experiments, analyzed data and commented on the manuscript;

A.J.B. designed and supervised the research, provided clinical care, collected and analyzed data, and drafted the manuscript.

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CD4+CD25+Fo xp3+ cells determined by a comprehensive DNA-based multiplex

prevent emergence of new CD25+ FOXP3+ lymphocytes after antigen


Table 1. Characteristics of 21 Transplant Recipients.

<table>
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<th>Value</th>
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<tr>
<td>Recipient age: years (range)</td>
<td>36 (13-56)</td>
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<tr>
<td>Donor age:</td>
<td>36 (16-58)</td>
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<tr>
<td>Sex</td>
<td>n (%)</td>
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<tr>
<td>Male</td>
<td>11 (52)</td>
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<tr>
<td>Female</td>
<td>10 (48)</td>
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<td>Disease distribution</td>
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<tr>
<td>CML</td>
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<tr>
<td>AML/MDS</td>
<td>3 (14)</td>
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<tr>
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<tr>
<td>Extensive</td>
<td>5 (24)*</td>
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<td>IST for cGVHD &gt;3 years at study</td>
<td>6 (29)</td>
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<tr>
<td>Follow-up period, years, median (range)</td>
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CML: chronic myelogenous leukemia; MDS: myelodysplastic syndrome; AML: acute myelogenous leukemia; ALL: MM: multiple myeloma; GVHD: graft versus host disease; cGVHD: chronic GVHD; IST: immunosuppressive therapy; * 1 recipient with active cGVHD at time of study.
Table 2. Quantitative Immunoglobulins and Lymphocyte Subsets in Transplant Recipients.

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<td>CD3+/CD56+ NK</td>
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FIGURE LEGENDS

Figure 1. Lymphocytes (T, B, NK, Treg, and Naïve T cells) in recipients and donors at transplant (15 recipient - donor pairs).

(A) Absolute total lymphocyte- (ALC), T-, B- and NK-cell counts.

(B) CD4+CD25+FOXP3+ regulatory T cells as a percentage of CD4+ T cells. (C) Paired analysis of naïve (CD27+CD45RO-CD57-) CD4+ and CD8+ T cells in recipients and donors at SCT. (D) Paired analysis of naïve CD4+ and CD8+ T cells in donors at SCT and donors in the second decade after SCT (9 pairs).

Figure 2. TREC analysis for thymic function.

(A) Correlation between the naïve CD4+ and CD8+ T cell numbers and TREC levels of recipients (■) and donors (▲) at transplant, with lower TREC levels in the recipients ($r^2 = 0.69, p = 0.01$) than that in the donors ($r^2 = 0.65, p = 0.02$).

(B) TREC levels of CD4+ and CD8+ T cells of recipients and donors at transplant (8 pairs).

Figure 3. Representative TCRVβ spectratype from a pair of recipient and donor pair at SCT. The spectratype profiles of the 23 Vβ subfamilies were analyzed. Shown are the CDR3 lengths on the x-axis versus the fluorescence intensity in the y-axis. R: recipient; D: donor. (A) CD4+ cells. (B) CD8+ cells.
Figure 4. TCRVβ repertoire complexity and diversity scores in recipients and donors. (A) An example of pattern diversity between recipient and donor with both gains and losses of repertoire peaks in the recipient. (B) The TCRVβ diversity (total complexity score) as a measure of the polyclonality of the CD4+ or CD8+ T-cell repertoire. Overall complexity within a Vβ subfamily was determined by counting the number of discrete peaks for a Vβ subfamily, with each subfamily graded on a score of 0 to 5. There was no significant difference in total complexity score of CD4+ (p = 0.30) or CD8+ (p = 0.64) T cells between recipients and donors at SCT (6 pairs). (C) There was no significant difference in total complexity score of CD4+ (p = 0.54) or CD8+ (p = 0.25) T cells between donors at SCT and donors in the second decade after SCT (4 pairs). (D) There are significant different diversity patterns in TCR Vβ subfamilies between recipients and donors at SCT (6 pairs). The median diversity score for CD4+ and CD8+ T cells was 18 ± 1.6 and 21 ± 0.8, respectively. (E) The median diversity score for CD4+ and CD8+ T cells was 10.2 ± 0.7 for both CD4+ and CD8+ T cells, between donors at SCT and donors in the second decade after SCT (4 pairs).
Figure 1

A. Absolute Cell Number/mL

B. Tregs % of CD4

C. Naïve T Cells %

D. Donor at SCT >10 years
Figure 3

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Figure 4

A

Identical GainLoss

Donor                             Recipient
ED

CD4                        CD8
Complexity Score
CD4               CD8
120
110
100
90
80
110
120

B

CD4               CD8
Recipient Donor Recipient Donor

C

CD4               CD8
SCT    >10 y   SCT    >10 y
Donor at SCT or at >10 years

D

CD4               CD8
Diversity Score

18 ± 1.6         21 ± 0.8

E

CD4               CD8
Diversity Score

10 ± 0.7         10 ± 0.7
Evolution of the donor T cell repertoire in recipients in the second decade after allogeneic stem cell transplantation