Lymphocyte reconstitution after allogeneic blood stem cell transplantation for hematologic malignancies

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Summary:

Forty-one patients were studied at set times after allogeneic blood stem cell transplantation (alloBSCT) for recovery of lymphocyte numbers and function. Cells were mobilized with G-CSF from HLA-matched related donors and cryopreserved. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine and methotrexate; G-CSF was administered post-transplant. Median time to absolute lymphocyte count (ALC) >500/μl was 17 days vs 41 and 49 days in historical alloBMT patients with G-CSF (n = 23) or no cytokine (n = 29) post-transplant, respectively (P,0.0001). CD4/CD8+ ratio was 1.9 on day 28 after alloBSCT, then gradually declined to 0.8 at 1 year due to more rapid CD8+ cell recovery. Mean phytohemagglutinin-induced T cell responses were lower than normal on day +28 (P < 0.05), then tended to recover towards normal values. Natural-killer cytotoxicity remained low from day +28 to 1 year post-alloBSCT, but considerable lymphokine-activated killer cytotoxicity was induced from cells already obtained on day +28. Faster lymphocyte recovery correlated with better survival in alloBSCT patients (median follow-up 287 days, P = 0.002), ALC recovery was not affected by acute GVHD, CMV infections or doses of infused cells. ALC recovery did not correlate with survival in either historical alloBMT group. These data suggest that after alloBSCT lymphocyte reconstitution is faster than after alloBMT, and that quicker lymphocyte recovery predicts better survival in the alloBSCT setting.

Keywords: stem cell transplantation; allogeneic; lymphocytes

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Allogeneic blood stem cells mobilized with growth factor are increasingly used for transplantation in patients with hematologic malignancies. Initial reports and subsequent retrospective comparisons have shown that allogeneic blood stem cell transplantation (alloBSCT) using cells from related HLA-identical donors is feasible, results in rapid and sustained hematopoietic recovery and is not associated with a significant increase in incidence of acute graft-versus-host disease (GVHD). However, the long-term safety and benefits of alloBSCT need to be confirmed in larger trials with longer follow-up.

Limited information is available regarding the nature and the clinical importance of immunological reconstitution after alloBSCT in humans. Infusion of larger numbers of cells and their exposure to growth factor during mobilization may result in faster and different immunologic reconstitution after alloBSCT as compared to conventional allogeneic bone marrow transplantation (alloBMT). The immune system is deeply involved in the pathophysiology of major complications that occur after allogeneic stem cell transplantation, including infections, graft-versus-host disease and relapse. Therefore studies of immune reconstitution, including lymphocyte reconstitution, could provide important data relative to the clinical outcome of alloBSCT. In the present report we analyzed the pattern of recovery of circulating lymphocytes, their functional status and the relationship between the lymphocyte recovery and survival after alloBSCT.

Methods

Patients and donors

Between December 1994 and April 1996, 41 patients with hematologic malignancies received HLA-matched related alloBSCT as part of a phase II trial at the University of Nebraska Medical Center (UNMC). This patient group was compared to two historical groups treated on sequential trials at the UNMC. The first comparison group (n = 23) received alloBMT between January 1994 and May 1995 followed by post-transplant granulocyte colony-stimulating factor (G-CSF) and the second group (n = 29) received alloBMT between January 1990 and April 1993 without post-transplant administration of cytokine.

Clinical characteristics of patients in all three groups are listed in Table 1. The studies were approved by the Institutional Review Board of UNMC.
Table 1  Patient characteristics

| BSCT | BMT (+G) | BMT (−G) |
|------|----------|----------|
| No. patients | 41 | 23 | 29 |
| Median age years (range) | 41 (21–63) | 38 (22–55) | 37 (19–57) |
| Female | 14 | 10 | 23 |
| Diagnosis: | | | |
| Acute myeloid leukemia | 8 | 6 | 12 |
| Acute lymphoid leukemia | 5 | 1 | 0 |
| Chronic myeloid leukemia | 12 | 10 | 11 |
| Chronic lymphocytic leukemia | 3 | 0 | 0 |
| Myelodysplastic syndrome | 1 | 3 | 5 |
| Hodgkin’s disease | 1 | 0 | 0 |
| non-Hodgkin’s lymphoma | 5 | 2 | 0 |
| Multiple myeloma | 6 | 1 | 1 |
| One HLA-antigen mismatch | 3 | 0 | 1 |
| Patient and donor CMV negative | 5 | 8 | 7 |
| Clinical outcomes (%): | | | |
| Cytomegalovirus infection | 16 (39) | 5 (22) | 9 (31) |
| Acute GVHD grade II–IV | 21 (51) | 10 (43) | 16 (55) |
| Acute GVHD grade III–IV | 10 (24) | 3 (13) | 9 (21) |
| Alive | 20 (49) | 15 (65) | 14 (48) |
| Relapse | 3 (7) | 3 (13) | 4 (14) |
| Median days to ANC >500/µl | 11 | 16 | 21 |
| Median follow-up in days | 287 | 574 | 1373 |

BSCT = blood stem cell transplantation; BMT (+G) = allogeneic bone marrow transplantation followed by G-CSF post-transplant; BMT (−G) = allogeneic bone marrow transplantation without post-transplant administration of cytokine; CMV = cytomegalovirus; ANC = absolute neutrophil count.

Preparative regimens and supportive care

Twenty-nine alloBSCT patients and all historical alloBMT patients received a preparative regimen consisting of etoposide 1800 mg/m² on day −7, cyclophosphamide 60 mg/kg/day on days −6 and −5, and total body irradiation (TBI) 200 cGy twice daily on days −3, −2 and −1 at a dose rate of 8–12 cGy/min. Four alloBSCT patients with history of prior radiotherapy received busulfan (16 mg/kg/4 days) instead of TBI; eight patients enrolled in the study protocol received cyclophosphamide and TBI without etoposide (n = 8). Post-transplant growth factor doses were identical for all patients; G-CSF 10 µg/kg/day was given by subcutaneous administration, starting 2 h after stem cell infusion until neutrophil recovery. GVHD prophylaxis consisted of cyclosporine (2 mg/kg intravenously every 12 h) starting on day −3 followed by oral cyclosporine (6.25 mg/kg every 12 h) when tolerated, and methotrexate (5 mg/m²) given intravenously on days +1, +3, +6 and +11. Patients with positive cytomegalovirus (CMV) cultures from bronchoalveolar lavage fluid, routinely performed on those seropositive for CMV or who received cells from CMV seropositive donors, received pre-emptive therapy with ganciclovir and intravenous immunoglobulin. Details of supportive care have been described previously.17

Stem cell collection and processing

Peripheral blood stem cells were mobilized from healthy donors with recombinant G-CSF (Amgen, Thousand Oaks, CA, USA) 5 µg/kg/day by subcutaneous administration for 5 days. Apheresis was begun on day 4 of G-CSF administration. A minimum of three daily 12-l collections was performed as part of a prospective trial of normal donor mobilization.18 The colony-forming unit granulocyte-macrophage (CFU-GM) content was analyzed using previously described methods.19 Leukapheresis was performed with a continuous flow blood cell separator (Cobe Laboratories, Lakewood, CO, USA) as previously described.17,20 Cells were cryopreserved in 5% dimethylsulfoxide (DMSO) plus 6% hydroxyethylstarch (McCaw, Irvine, CA, USA) in a mechanical −135°C freezer.21 Allograft product characteristics are shown in Table 2. For alloBMT, marrow was harvested from the posterior iliac crests and was infused fresh.17 Day 0 for the recipient was defined as the day of bone marrow or blood stem cell infusion.

Collection of peripheral blood mononuclear cells

Peripheral blood samples were procured from apheresis collections, and from patients following alloBSCT or alloBMT on days +28, +100 and 1 year post-transplant. Blood samples were obtained from six unrelated healthy volunteers and used as normal controls in the cytotoxicity and mitogen assays. Peripheral blood mononuclear cells (PBMC) were separated over a separation gradient using standard methods.20

Flow cytometric phenotyping of PBMC was performed using standard whole blood technique and a Coulter multi-Q Prep.18,20 All antibodies were directly conjugated to fluorescein, phycoerythrin, or cychrome. Antibodies utilized included: CD3 (T11 or G1), CD4 (T4), CD8 (T8), CD19 (B4), CD25 (IL-2R1), CD45 (56), CD56 (NKH-1), CD45RA (2H4), (Coulter, Miami, FL, USA), and CD34 (HPCA-2) (Becton Dickinson, San Diego, CA, USA).
Table 2  Cellular characteristics of infused allogeneic stem cell products obtained from healthy donors

| No. | Median | Range       |
|-----|--------|-------------|
| G-CSF mobilized blood cells |
| Nucleated cell dose (10^9/kg) | 41 | 10.43 | 7.12–17.69 |
| CFU-GM dose (10^6/kg) | 41 | 45.56 | 6.89–171.72 |
| CD34^+ dose (10^6/kg) | 41 | 7.87 | 1.56–37.89 |
| Total lymphocytes infused (×10^9) | 41 | 55.14 | 29.2–111.5 |
| CD3^+ cells (10^8/kg) | 41 | 7.09 | 3.4–13.01 |
| CD3^+/CD4^+ cells (10^8/kg) | 41 | 4.48 | 0.74–8.98 |
| CD3^+/CD8^+ cells (10^8/kg) | 41 | 2.22 | 1.18–5.73 |
| CD4^−/CD56^+ cells (10^6/kg) | 33 | 0.61 | 0.7–2.44 |
| Bone marrow harvest BMT (+G) |
| Nucleated cell dose (10^9/kg) | 23 | 2.60 | 2.0–3.16 |
| CFU-GM dose (10^6/kg) | 23 | 5.73 | 1.67–23.7 |
| Bone marrow harvest BMT (−G) |
| Nucleated cell dose (10^9/kg) | 29 | 2.84 | 1.21–4.14 |
| CFU-GM dose (10^6/kg) | 29 | 8.31 | 1.63–19.03 |

All characteristics were determined on fresh cells. CD34 and lymphocyte studies were performed only on allogeneic blood cell products.

BMT (+G) = group which received G-CSF after BMT; BMT (−G) = group which did not receive growth-factor after BMT.

Mitogen assay

One hundred thousand cells/well in 96-well microtiter plates were incubated with mitogen phytohemagglutinin (PHA) 2.5 μg/ml, or media alone for 72 h at 37°C. Eighteen hours prior to harvesting, 1 μCi of 3H-thymidine was added. Cells were harvested using a PHD cell harvester (Cambridge Technology, Boston, MA, USA). The radioactivity in the cell harvest was counted using a Beckman liquid scintillation counter (Chicago, IL, USA).

Cell culture

The human chronic myeloid leukemia cell line, K562 (NK-sensitive), and the human B cell lymphoma cell line, Raji (NK-resistant), were grown in vitro in 25-cm² tissue culture flasks in RF10 medium. K562 and Raji cells were labeled with chromium-51 (Na₂¹⁵⁵CrO₄) (Amersham, Arlington Heights, IL, USA), and used as targets for natural killer (NK) and lymphokine-activated killer (LAK) cytotoxicity assays.

Generation of lymphokine-activated cytotoxic cells

The baseline cytotoxicity of fresh PBMNC from patients, normal volunteers, and from G-CSF mobilized blood products was determined against K562 cells (NK-sensitive) or against Raji cells (NK-resistant). Additionally, PBMNC were incubated in vitro with interleukin-2 (IL-2; Chiron, Emeryville, CA, USA) and tested for cytotoxicity against K562 or against Raji cells (LAK activity). Five million mononuclear cells were cultured in T-25 flasks containing 5 ml RPMI 1640 medium supplemented with 10% fetal calf serum, 1-glutamine (2 mm), penicillin (100 U/ml), streptomycin (100 μg/ml), 50 μM β-mercaptoethanol, and IL-2 1000 U/ml for 72 h at 37°C in 5% CO₂ and 95% air. Following the incubation period, cells were harvested and assayed for their cytotoxicity.

Cytotoxicity assay

Both the ⁵¹Cr-labeled targets and effector cells were resuspended in RF10 medium to yield effector:target cell ratios of 100:1, 50:1, 25:1, and 12.5:1, mixed in round bottom 96-well microtiter plates and incubated for 4 h at 37°C. At the end of the incubation period the plates were centrifuged for 10 min at 400 g, and 150 μl of supernatant was removed from each well. Radioactivity was counted using a Beckman 5500 gamma counter. The percentage of target cells lysed was calculated as previously described. The results are presented as the mean percent of lysis from the triplicate cultures.

Other evaluations and definitions

Lymphocyte and neutrophil recovery rates were defined as the time to first of 3 consecutive days with absolute lymphocyte count (ALC) or absolute neutrophil count (ANC) >500/μl. Time to monocyte recovery was the number of days after transplant required to achieve monocyte count >100/μl. Other clinical endpoints were: overall survival, acute GVHD grade II–IV or III–IV, and documentation of CMV infections. Identification of acute GVHD required biopsy confirmation and was graded clinically according to published criteria.

Statistical analysis

Difference between distributions was analyzed using the Wilcoxon rank-sum test or Kruskal–Wallis analysis of variance. Comparisons of proportions were done using Fisher’s exact test. Distribution of the time-to-event data was estimated using the Kaplan–Meier method. Time-to-event data were analyzed using the log rank test.
Results

Lymphocyte recovery

Thirty-three patients after alloBSCT, 19 after alloBMT with post-transplant growth factor (+G), and 22 after alloBMT without post-transplant growth factor (−G) achieved ALC ≥500/μl. The median times to ALC ≥500/μl was 17 days (9–55) after alloBSCT, 41 days (14–399) after alloBMT (+G), and 49 days (14–475) after alloBMT (−G), (P < 0.0001) (Figure 1).

Median numbers of CD3+/CD8+ cells, and CD56+ cells were within normal reference limits by day +28 after alloBSCT (Figure 2a). Median numbers of CD3+ and CD3+/CD4+ cells reached the normal reference range at 1 year post-transplant. Early after alloBSCT, absolute numbers of CD3+/CD8+ cells were higher than CD3+/CD8− cells which resulted in a normal CD4/CD8 ratio of 1.9 (0.4–6.2) by day +28 (P = 0.043). Absolute numbers of CD3+/CD4+ cells increased more slowly thereafter and the median CD4/CD8 ratio dropped to 1.1 (0.2–5.6) by day +100 and to 0.8 (0.3–2.9) 1 year post-transplant. Median percentage of CD4+ cells with the phenotype of naive CD45RA− cells increased more slowly thereafter and the median CD4/CD8 ratio dropped to 1.1 (0.2–5.6) by day +100 and to 0.8 (0.3–2.9) 1 year post-transplant. Median percentage of CD4+ cells with the phenotype of naive CD45RA− T lymphocytes remained below the normal range throughout the follow-up. Absolute numbers of CD19+ cells remained decreased during the first year after alloBSCT.

Historical immunophenotyping data were available only for the alloBMT (−G) group, at +100 days and at +1 year time-points (Figure 2b). AlloBSCT patients had higher median numbers of circulating CD3+ cells, CD3+/CD4+ cells, these differences approached statistical significance at 1 year post-transplant (respective P values were 0.028 and 0.063). The recovery kinetics of CD3+/CD8+ cells, CD56+ cells, and CD19+ cells were similar between alloBSCT and alloBMT groups. On day 100 and 1 year after alloBMT median CD4/CD8 ratios were 0.5 (0.1–1.6) and 0.4 (0.1–1.4) respectively.

Figure 1 Actuarial time to recovery of ≥500/μl lymphocytes (ALC) in patients after alloBSCT (n = 41), alloBMT followed by G-CSF (alloBMT +G), (n = 23) or without post-transplant growth factor (alloBMT −G); (n = 29).

Figure 2 Median numbers of lymphocyte subsets after (a) allogeneic blood stem cell transplantation, and (b) in historical bone marrow transplantation patients. Allogeneic BMT group included only patients that did not receive G-CSF post-transplant. ND = not done; * = instances where comparisons of alloBMT data with alloBSCT results showed P values less than 0.10 (Wilcoxon rank-sum test).

Figure 3 Phytohemagglutinin (2.5 μg/ml) induced T cell proliferative responses from cells obtained after allogeneic blood stem cell transplantation. Media only (n = 9), day +28 (n = 9), day +100 (n = 4), 1 year post-transplant (n = 6). G-CSF mobilized normal donor stem cells (n = 22), and healthy volunteers (n = 6). Confidence intervals represent standard error of the mean. Results are expressed as counts per minute/10⁶ cells. * = P value <0.05 when compared to healthy volunteers.
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Figure 4  Natural killer and lymphokine-activated killer (LAK) cytotoxicity after allogeneic blood stem cell transplantation. Columns indicate data from G-CSF-mobilized normal donor blood stem cell collections (n = 54), normal volunteer PBMNC (n = 6), patients PBMNC obtained on days +28 (n = 26), +100 (n = 21), and 1 year (n = 8) post-transplant. (a) Baseline NK cytotoxicity of fresh PBMNC; (b) baseline cytotoxicity of fresh PBMNC against Raji targets; (c) LAK cytotoxicity against K562 targets after 72 h in vitro incubation with IL-2 (1000 U/ml); (d) LAK cytotoxicity against Raji targets after 72 h in vitro incubation with IL-2 (1000 U/ml). Results are expressed as the mean percent of cytotoxicity at the effectortarget (E:T) ratio 50:1. Confidence intervals represent ± standard error of the mean.

Recovery of T cell function
Mean phytohemagglutinin-induced T cell proliferation was only 46% of normal on day +28 (P < 0.05), percentages of mean normal values on days +100, and 1 year post-transplant were 72% and 81%, respectively. However, day 28, day 100, and 1 year proliferation levels were not statistically different from each other (Figure 3).

Recovery of cytotoxic function
Mean circulating NK cytotoxicity and cytotoxicity against Raji cells were low (less than 10% of lysis) during the first year after alloB SCT (Figures 4a and b). However, after PBMNC were in vitro incubated with IL-2, mean cytotoxicity levels increased (Figures 4c and d). Comparable results were obtained when the Daudi cell line was used instead of Raji cells as an NK-resistant tumor target (data not shown). Of interest, cytotoxicity levels were consistently different (usually lower) in control cultures obtained by pheresis compared to cultures with normal donor cells (Figures 4a–d).

Clinical correlations
To assess the clinical relevance of lymphocyte recovery, time to achieve ALC ≥500/μl was correlated with survival after alloB SCT. Overall survival was better for half of alloB SCT patients who achieved ALC ≥500/μl within 17 days post-transplant, than for the other half who required more time; 1 year probabilities of survival were 79% and 19%, respectively (P = 0.0024) (Figure 5a). Three patients who died after alloB SCT were not eligible for survival analysis (two early deaths and one with CLL whose ALC never dropped below 500/μl). There were six deaths in the group with faster ALC recovery (four acute GVHD, two chronic GVHD), and 12 deaths in the group with slower ALC recovery (three relapse, three aspergillus infection, two ARDS, two acute GVHD, one multiorgan failure,
one adenovirus pneumonia). More severe grades of acute GVHD (grades III–IV) were associated with poorer survival \((P = 0.02)\). Age, incidence and severity of acute GVHD, CMV infections, or doses of infused cells did not have an impact on the rate of lymphocyte recovery after alloBSCT (Table 3). Onset of acute GVHD or CMV infections did not have impact on the recovery of analyzed T cell subsets or CD56+ cells \((P\) values 0.13–1.00). Slower lymphocyte recovery after alloBSCT was not the consequence of steroid therapy for GVHD, as only three patients were started on high-dose steroids for acute GVHD treatment prior to achieving an ALC \(\geq500/\mu l\) (on days 15, 19 and 36 post-transplant).

In contrast to alloBSCT, earlier than median time for achieving ALC \(\geq500/\mu l\) was not predictive for better survival in either of the historical alloBMT groups (Figures 5b and c). Poorer outcome among patients with slower lymphocyte recovery after alloBSCT was not a consequence of absolutely slower lymphocyte reconstitution; median time for achieving ALC \(\geq500/\mu l\) in the group of patients with slower lymphocyte recovery after alloBSCT was 35 days, significantly faster than in corresponding alloBMT groups (84 days after alloBMT (+G), \((P = 0.012)\), and 165 days after alloBMT (−G), \((P = 0.0005)\)).

To study the relationship between recovery of other hematopoietic lineages and survival after alloBSCT, rates of monocyte and neutrophil recovery were analyzed (both medians were at 11 days post-transplant). Faster than median rates of achieving \(\geq100/\mu l\) monocytes, or \(\geq500/\mu l\) neutrophils were also associated with better survival \((P\) values were 0.018 and 0.051 for monocyte and neutrophil recovery, respectively). No such correlations were found in either of the two historical alloBMT groups (data not shown).

Discussion

The first indication that the source of stem cells may affect the rate of lymphocyte reconstitution came from autologous blood stem cell transplantation (autoBSCT) studies.\(^{24,25}\) Compared to autoBMT and alloBMT, autoBSCT resulted in faster lymphocyte and T cell subset recovery, which was explained by the greater number of lymphocytes and other progenitors infused with blood stem cell products. AlloBSCT is associated with an infusion of significantly larger numbers of growth factor-exposed lymphocytes and other cells, which may affect immunological reconstitution and/or the incidence of clinical complications.\(^{11–14}\) These putative effects could be mediated by higher frequencies of a particular cell subtype, by a different functional status of infused cells, by a higher level or a different pattern of post-transplant cytokine production, or by combination of these factors.\(^{12–14}\)

In the present report, patients who received alloBSCT had a significantly faster lymphocyte recovery than patients who received alloBMT. Recovery of lymphocyte subsets in the alloBSCT group was characterized by an initially more rapid increase of CD3+/CD4+ cells than CD3+/CD8+ cells. These findings are in contrast to alloBMT data where CD4+ T cells typically recover more slowly, resulting in consistently lower CD4/CD8 ratios, post-transplant.\(^{7,11,16,26}\) Rapid recovery of CD4+ cells early after alloBSCT may be a reflection of cellular and biological characteristics of the normal donor blood, in contrast to normal bone marrow where the CD4/CD8 ratio is typically less than 1.0.\(^{27}\)

An alternative explanation could be more rapid thymic differentiation after alloBSCT.\(^{28}\) In the current study, the number of naive post-thymic CD4+/CD45RA+ T cells remained low, similar to observations after alloBMT in adults, suggesting that a more rapid thymic differentiation of CD3+/CD4+ cells in adult alloBSCT recipients was not the case.\(^{29,30}\)

Mitogen-induced T cell proliferation is considered to be a good in vitro correlate of clinical immunological recovery.\(^{13}\) Very low mitogen-induced T cell responses are typically present during the first 6 to 12 months after alloBMT, reflecting a low frequency of responding precursor cells.\(^{16,26,31–34}\) In the current study, PHA-induced T cell pro-

### Table 3 Factors affecting lymphocyte recovery or survival in patients after allogeneic blood stem cell transplantation

| Age (years) | Days to achieve ALC \(>500/\mu l\) | \(P\) | Median survival (months) | \(P\) |
|---|---|---|---|---|
| <41 | 17 | 0.84 | NR | 0.26 |
| >41 | 16 | 5 | | |
| Acute GVHD | | | | |
| Grade II–IV | 16 | 0.90 | 6 | 0.84 |
| Grade 0–I | 19 | 5 | | |
| Acute GVHD | | | | |
| Grade III–IV | 19 | 0.56 | 3 | 0.02 |
| Grade 0–II | 16 | 16 | | |
| CMV infection | | | | |
| Yes | 14 | 0.58 | 5 | 0.69 |
| No | 19 | 5 | | |
| Above or below median doses of CD3, CD4, CD8, CD56, MNC, CD34, CFU-GM or total lymphocytes | — | 0.21–0.94 | — | 0.23–0.92 |

\(P\) values from log-rank test.

NR = median survival not reached.
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Figure 5 Probability of overall survival in patients with different rates of achieving absolute lymphocyte count of >500/μl after (a) allogeneic blood stem cell transplantation; (b) allogeneic BMT followed by G-CSF (+G); and (c) after allogeneic BMT with no post-transplant cytokine (−G). Patients were divided into those who had time for ALC recovery slower than median number of days and in those who had time to absolute lymphocyte count >500/μl faster than median number of days.

Lymphocyte reconstitution started to recover quickly after alloBSCT, a finding also reported by Ottinger et al.13 In the present report post-alloBSCT NK cytotoxicity remained persistently low, despite a high number of circulating CD56+ cells. This sharply contrasts with most alloBMT studies where NK cytotoxicity was one of the first cellular functions to recover.25,34–38 The use of G-CSF for mobilization or post-transplant could have modified the differentiation of regulatory cells resulting in a low level of circulating NK cytotoxicity.13,14 G-CSF effects could also be accountable for observed differences in NK and LAK cytotoxicity between mobilized cells and normal donor controls. IL-2-responsive cells that can generate LAK cytotoxicity were present in the circulation early after alloBSCT, similar to published results after alloBMT.36,39,40 Some studies have suggested the ominous predictive value of an early drop in lymphocyte counts after alloBMT.33,41,42 In the present analysis, there was no correlation between the rate of lymphocyte recovery and survival after alloBMT. In contrast, a strong positive correlation between earlier lymphocyte recovery and overall survival was observed after alloBSCT. The exact reason for this association is not clear. Impaired lymphocyte recovery did not correlate with onset or severity of acute GVHD. The main causes of death among alloBSCT patients with slower lymphocyte reconstitution were infections or transplant-related toxicity. Systemic inflammation that occurs after increased regimen-induced toxicity is usually followed by a compensatory cytokine-mediated anti-inflammatory response that causes immunosuppression and susceptibility to infections.43,44 In the alloBSCT setting, lymphocyte recovery occurs 3–4 weeks earlier than after BMT, during a period when the effects of compensatory immunosuppressive response may still be present. In this scenario, slower lymphocyte recovery after alloBSCT could be a surrogate marker for higher regimen-related toxicity.45 Finally, faster lymphocyte recovery may be a sign of more rapid immunological reconstitution, resulting in fewer infections, and fewer deaths.11 The duration of this study was not sufficient to allow an evaluation of the impact that chronic GVHD might have on immunological reconstitution in alloBSCT patients.46 Our results must be interpreted relative to the particular clinical protocol used, and confirmatory studies are needed. If the time to lymphocyte recovery is an independent prognostic factor for survival after alloBSCT, stimulating post-transplant lymphopoiesis could improve patient outcome.47

The data presented here suggest that after alloBSCT, recovery of lymphocytes and certain lymphocyte functions occur early, and that alloBSCT patients with faster lymphocyte recovery may have better short-term survival. More detailed knowledge of alloBSCT biology, as well as prospective randomized clinical studies are needed to identify critical components of the allograft and the immune system that may determine outcome after alloBSCT.

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