

Different Immune Reconstitution between Cord Blood and unrelated Bone Marrow Transplantation with Relation to Chronic Graft-versus-Host Disease

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ABSTRACT

Background: Advances of allogeneic hematopoietic cell transplantation (allo-HCT) have brought long-term survival to the patients with hematologic malignancies. Chronic graft-versus-host disease (GVHD) is one of major problems for the long-survivors after allo-HCT. Dysregulation of immune reconstitution has been reported to be involved in the pathogenesis of chronic GVHD. Differences of immune reconstitution between cord blood transplantation (CBT) and unrelated bone marrow transplantation (uBMT) remain unclear in long-term survivors. We investigated immune reconstitution in patients who survive for more than 2 years after CBT (n=21) or uBMT (n=20) without relapse of underlying disease.

Materials and Methods: Using flowcytometric analysis of peripheral blood, we investigated immune reconstitution of T cells, B cells, and NK cells between CBT and uBMT patients. We collected clinical data regarding allo-HCT and examined the relation of immune reconstitution to the development of chronic GVHD.

Results: Between CBT and uBMT patients, we found significant differences in absolute cell number of CD8+ as well as CD19+ cell and CD4/CD8 ratio even more than 2 years after allo-HCT. Among uBMT patients, absolute cell number of naïve CD4+ cell was significantly lower in patients with chronic GVHD. In addition, we found significant differences in absolute cell number of CD19+ cell, especially naïve B cell between patients with and without chronic GVHD in both CBT and uBMT patients.

Conclusion: These results suggest that differences of immune recovery between CBT and uBMT patients may exist even in patients who survive for more than 2 years and might be related to the development of chronic GVHD.

Keywords: Immune reconstitution; Cord blood transplantation; Unrelated bone marrow transplantation; Chronic GVHD

INTRODUCTION

Allogeneic hematopoietic cell transplantation (allo-HCT) has improved the prognosis of the patients with hematologic malignancies. Advances of conditioning regimens and immunosuppressive therapy contribute to the improvement of the prognosis. In

addition, the introduction of unrelated bone marrow transplantation (uBMT) and cord blood transplantation (CBT) increased the chance to receive allo-HCT. These progresses have led to the result that some patients survive for more than a decade. Concomitantly, there have increased various

problems to disturb the quality of life of long-term survivors after allo-HCT^{1, 2}.

Chronic graft-versus-host disease (GVHD) is one of major problems for long-term survivors after allo-HCT, leading to reduced patient-reported quality of life and non-relapse mortality³⁻⁵. Risk factors for chronic GVHD include prior acute GVHD, donor peripheral blood stem-cell grafts, HLA disparity, female donors for male recipients, and recipient age⁶. Concerning the graft of allo-HCT, the incidence and severity of chronic GVHD are recently reported to be lower in CBT than uBMT patients⁷.

Acute GVHD is thought to be mediated primarily by mature donor T cells in the allogeneic stem cell product. By contrast, chronic GVHD is now considered to be more complex immune reaction. Both donor-derived effector T and B cells contribute to the pathology of chronic GVHD^{8, 9}. In addition, regulatory elements within T and B cell lineages play important roles in the development and maintenance of immune tolerance after allo-HCT^{10, 11}. Several reports have shown that the differences in immune reconstitution exist between the patients who received cord blood and other hematopoietic stem cell sources. In CBT patients, delayed recovery of T cells has been reported, by contrast, B cell numbers were higher compared to the patients received HLA-matched sibling or unrelated peripheral blood stem cell transplantation¹²⁻¹⁴. However, the observation duration was up to 2 years post allo-HCT. The differences in immune reconstitution more than 2 years after allo-HCT between CBT and uBMT have not been elucidated. In this study, we investigated the differences of immune reconstitution between CBT and uBMT patients, who survive for more than 2 years after allo-HCT without relapse of underlying disease, in relation to the development of chronic GVHD in our institute.

MATERIALS AND METHODS

Study design

To determine whether the differences in immune reconstitution would exist between CBT and uBMT patients in long-survivors, we selected patients who had attended to our outpatient clinic for more than

2 years after allo-HCT and showed no symptoms of infections and relapse of underlying disease. Twenty-one patients who had received CBT (CBT group) and 20 patients who had received HLA-matched uBMT (uBMT group) from January 2002 to January 2014 were enrolled in this study. We collected peripheral blood for flowcytometric analysis to investigate immune reconstitution and clinical symptoms regarding allo-HCT at the time after allo-HCT described in duration in Table 1 after informed consent was given. We examined whether the differences in the immune reconstitution between CBT and uBMT patients who survive for more than 2 years after allo-HCT and any relations to the development of cGVHD could exist. This study was approved by the ethical committee of our institute.

Patient characteristics

Patient characteristics concerning allo-HCT are listed in Table 1. Median age in CBT group (45 years old, range: 24-63) was significantly older than in uBMT group (38 years old, range: 20-56) ($p < 0.01$). But there was no significant difference in median duration from allo-HCT to the time, when the investigation was performed, between CBT (5.5 years, range: 2.4-13.1) and uBMT group (7.3 years, range: 2.1-14.1). Most of underlying disease were hematologic malignancies, only one patient in uBMT group was non-malignant disease (paroxysmal nocturnal hematuria/ aplastic anemia).

All of CBT were performed using cord blood which matched a minimum of 4 of 6 HLA loci to the patient at antigen level for HLA-A, HLA-B, and HLA-DRB1. In uBMT group, all patients received bone marrow but not peripheral blood stem cell from HLA-matched unrelated donor.

In 9 patients in CBT group and 12 patients in uBMT group, cyclophosphamide (60mg/kg x2) and total body irradiation (TBI) (3Gy x4) had been performed as conditioning regimen (myeloablative conditioning; MAC) (Table 1). In 12 patients in CBT group and 8 patients in uBMT group, various reduced intensity conditioning (RIC) had been conducted (Table 1). For the prophylaxis of GVHD, calcineurine inhibitor plus short-term methotrexate had been introduced into all of patients.

Table 1. Clinical data in the patients who had received cord blood transplantation (CBT) and unrelated bone marrow transplantation (uBMT)

| CBT | | | | | | | | |
|---------|----------------|-----------------|--------|--------|------------|----------------|------------------|--------------------------------|
| Disease | Disease status | Age at allo-HCT | Gender | Source | CMV status | Conditioning | GVHD prophylaxis | Duration (years post allo-HCT) |
| AML | NC | 57 | F | CB | + | FLU+Mel+CA+TBI | CsA+sMTX | 2.8 |
| ATLL | CR1 | 57 | M | CB | + | FLU+Mel+TBI | CsA+sMTX | 3.4 |
| Ph+ALL | CR2 | 48 | M | CB | + | FLU+BU4+TBI | CsA+sMTX | 2.4 |
| DLBCL | CR2 | 51 | F | CB | + | FLU+Mel+TBI | CsA+sMTX | 2.4 |
| CMMoL | NC | 40 | M | CB | + | CY+TBI | CsA+sMTX | 3.9 |
| Ph+ALL | CR2 | 44 | M | CB | + | CY+TBI | CsA+sMTX | 6.1 |
| FL | NC | 56 | M | CB | + | FLU+Mel+TBI | CsA+sMTX | 7.9 |
| ALL | CR1 | 32 | F | CB | + | CY+TBI | CsA+sMTX | 9.5 |
| FL | CR3 | 51 | M | CB | + | FLU+Mel+TBI | CsA+sMTX | 5.5 |
| ALL | CR1 | 41 | M | CB | + | FLU+Mel+TBI | CsA+sMTX | 4.0 |
| AML | CR1 | 45 | M | CB | + | CY+AraC+TBI | CsA+sMTX | 7.3 |
| AML | CR2 | 45 | F | CB | + | FLU+BU+TBI | CsA+sMTX | 3.0 |
| Ph+ALL | CR1 | 39 | F | CB | - | CY+TBI | CsA+sMTX | 10.8 |
| AML | CR2 | 44 | F | CB | + | CY+TBI | CsA+sMTX | 7.1 |
| PTCL | NC | 33 | M | CB | + | CY+TBI | CsA+sMTX | 7.6 |
| AML | CR1 | 52 | F | CB | + | FLU+Mel+TBI | CsA+sMTX | 3.5 |
| AML | CR1 | 38 | F | CB | + | CY+TBI | CsA+sMTX | 2.1 |
| CML | CP1 | 24 | M | CB | + | CY+TBI | CsA+sMTX | 13.1 |
| ATLL | CR1 | 41 | F | CB | + | FLU+Mel+TBI | CsA+sMTX | 3.1 |
| MDS | RA | 62 | M | CB | + | FLU+CA+Mel+TBI | CsA+sMTX | 6.3 |
| Ph+ALL | CR1 | 63 | M | CB | + | FLU+Mel+TBI | CsA+sMTX | 6.2 |
| uBMT | | | | | | | | |
| AML | CR1 | 25 | F | uBM | + | CY+TBI | CsA+sMTX | 8.8 |
| MDS | RAEB | 54 | M | uBM | + | CY+TBI | CsA+sMTX | 7.8 |
| AML | CR1 | 34 | M | uBM | + | CY+TBI | CsA+sMTX | 3.4 |
| ALCL | CR2 | 56 | M | uBM | + | FLU+Mel+TBI | CsA+sMTX | 4.3 |
| AML | CR2 | 48 | F | uBM | - | FLU+BU4+TBI | CsA+sMTX | 5.0 |
| MM | sCR | 39 | F | uBM | - | FLU+Mel+TBI | CsA+sMTX | 2.1 |
| DLBCL | CR2 | 44 | F | uBM | + | FLU+BU2 | CsA+sMTX | 6.8 |
| FL | CR2 | 46 | F | uBM | + | FLU+Mel | CsA+sMTX | 9.3 |
| MDS | RAEB2 | 26 | M | uBM | - | CY+TBI | CsA+sMTX | 10.3 |
| AML | CR1 | 26 | F | uBM | + | CY+TBI | CsA+sMTX | 13.6 |
| MDS | RAEB | 38 | M | uBM | + | CY+TBI | CsA+sMTX | 6.2 |
| AML | CR1 | 38 | F | uBM | + | CY+TBI | CsA+sMTX | 8.0 |
| PNH/AA | | 39 | F | uBM | + | CY+FLU+ATG | CsA+sMTX | 3.8 |
| AML | CR1 | 49 | M | uBM | + | CY+TBI | CsA+sMTX | 10.2 |
| MDS | RAEB2 | 29 | M | uBM | + | CY+TBI | CsA+sMTX | 8.0 |
| CML | BC | 35 | F | uBM | + | CY+TBI | CsA+sMTX | 11.7 |
| NHL | CR2 | 32 | M | uBM | + | CY+TBI | CsA+sMTX | 14.1 |
| CML | CR2 | 38 | M | uBM | + | CY+TBI | CsA+sMTX | 6.6 |
| AML | CR2 | 20 | F | uBM | + | FLU+BU2+TBI | Tac+sMTX | 2.2 |
| DLBCL | NC | 48 | F | uBM | + | FLU+BU2+TBI | CsA+sMTX | 2.2 |

Allo-HCT; allogeneic hematopoietic cell transplantation, CMV; cytomegalovirus, GVHD; graft-versus-host disease, AML; acute myelogenous leukemia, ATLL; adult T cell leukemia/lymphoma, Ph+ALL; Philadelphia chromosome positive acute lymphoblastic leukemia, DLBCL; diffuse large B cell lymphoma, CMMoL; chronic myelomonocytic leukemia, FL; follicular lymphoma, ALL; acute lymphoblastic leukemia, PTCL; peripheral T cell lymphoma, CML; chronic myelogenous leukemia, MDS; myelodysplastic syndrome, ALCL; anaplastic large cell lymphoma, MM; multiple myeloma, PNH/AA; paroxysmal nocturnal hematuria/aplastic anemia, NHL; non-Hodgkin lymphoma, NC; non-complete remission, CR1; first complete remission, CR2; second complete remission, CR3; third complete remission, RA; refractory anemia, RAEB; refractory anemia with excess blast, sCR; stringent complete remission, BC; blastic crisis, CB; cord blood, uBMT; unrelated bone marrow transplantation, FLU; fludarabine, Mel; melphalan, CA; cytosine-arabincytoside, TBI; total body irradiation, BU; busifan, CY; cyclophosphamide, CsA; cyclosporine A, sMTX; short-term methotrexate.

Diagnosis of acute and chronic GVHD

We collected clinical data from medical charts and diagnosed acute and chronic GVHD, according to the criteria previous described¹⁵.

Flowcytometry

Aliquots of whole peripheral blood were stained with various mAbs, according to manufactured instructions. We had performed three color flowcytometric analysis. mAbs using in this study were as following. PC5-conjugated anti-CD4 mAb, PC5-conjugated anti-CD8 mAb, PE-conjugated anti-CD45RO mAb, FITC-conjugated anti-CD25 mAb, PE-conjugated anti-CD127 mAb, PC5-conjugated anti-CD19mAb were purchased from Beckman Coulter. FITC-conjugated anti-CD27 mAb and PE-conjugated anti-IgM mAb were purchased from Pharmingen.

In CD4+ or CD8+ cells, CD27+CD45RO- cells have been designated as naïve T cell (Figure 1A), and CD25+CD127-~+ cells have been designated as regulatory T cell¹⁶ (Figure 1B). In CD19+ cells, CD27- cells have been designated as naïve B cell and CD27+ B cells as memory B cell (Figure 1C). CD3-CD56+ cells have been determined as NK cell.

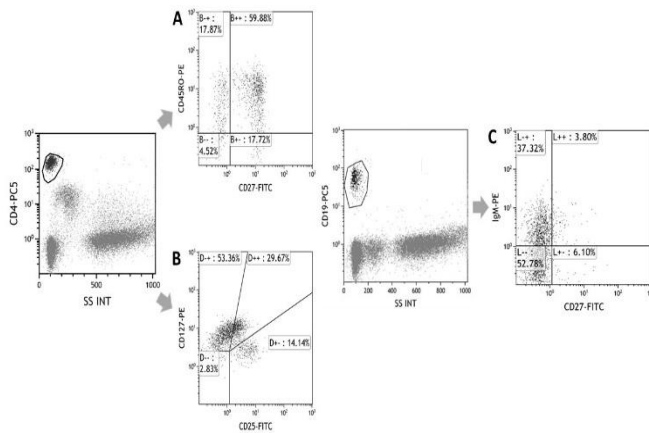


Figure 1.

Figure 1. Representative data of flowcytometric analysis of the peripheral blood

After gating CD4+ cells, we investigated the expression of CD45RO and CD27 (A). Similar analysis had been done in CD8+ cells. In addition, CD25+CD127negative to weekly positive cells in CD4+ cells were detected as regulatory T cells (B). In CD19+ cells, we investigated the expression of CD27 and IgM (C).

Statistical Analysis

Immunologic parameters, described in Flowcytometry, were compared between the patients who received CBT and uBMT. In some investigation, immunologic parameters of the patients without versus with chronic GVHD were compared. Comparison between 2 groups was performed with Mann-Whitney U test.

RESULTS

Reconstitution in T cell between CBT and uBMT group

We first examined the differences in the reconstitution of T cells between CBT and uBMT group. As shown in Figure 2A, there was no significant difference in absolute number of CD4+ T cell, but significant differences in absolute number of CD8+ cell and CD4/CD8 ratio between two groups. Absolute cell number of CD8+ cell was significantly higher in uBMT patients (Figure 2B). As a result, CD4/CD8 ratio was significantly lower in uBMT patients (Figure 2C). We next examined the ratio and cell count of subpopulations in T cells. In CD4+ and CD8+ cells, no significant difference in ratio and absolute cell number of naïve cell (CD27+CD45RO-) was observed between two groups as well as memory cell (CD27-CD45RO+) (data not shown). Furthermore, we have observed no significant difference absolute cell number of regulatory T cell, which has been reported to be involved in the pathogenesis of chronic GVHD (Figure 2D).

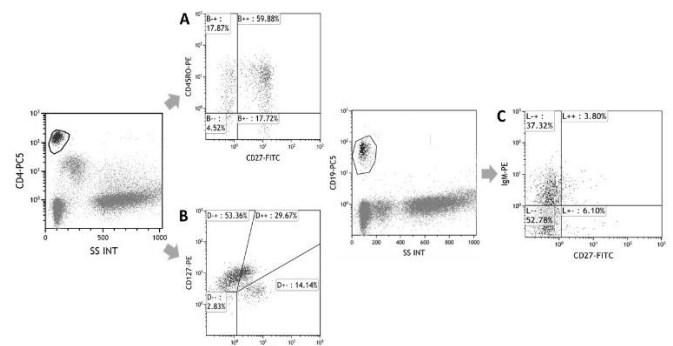


Figure 1.

Figure 2. Differences in T cell recovery between CBT and uBMT group. Absolute count of CD4+ cell (A), CD8+ cell (B), CD4/CD8 ratio (C), and regulatory T cell (D) of peripheral blood were compared between CBT and uBMT group. * indicates differences that are statistically significant ($p < 0.05$).

Reconstitution in B cell and NK cell

We next examined the recovery of B cells. As shown in Figure 3A, absolute cell number of CD19+ cell in uBMT group was significantly lower than in CBT group ($p < 0.05$), although there was no significant difference in serum levels of immunoglobulins (IgG, IgA, and IgM) (data not shown). By contrast, no significant difference was observed in NK cell counts between two groups (Figure 3B). Absolute cell number of naïve B cell (CD19+CD27-) was also lower in uBMT group without statistical significance ($P = 0.05$) (Figure 3C). In memory B cells (CD19+CD27+), absolute cell number tended to be lower in uBMT than CBT group without statistical significance (Figure 3D, $p = 0.10$). These results suggest that the differences in the reconstitution of B cells between CBT and uBMT patients at a long-term period after allo-HCT could exist.

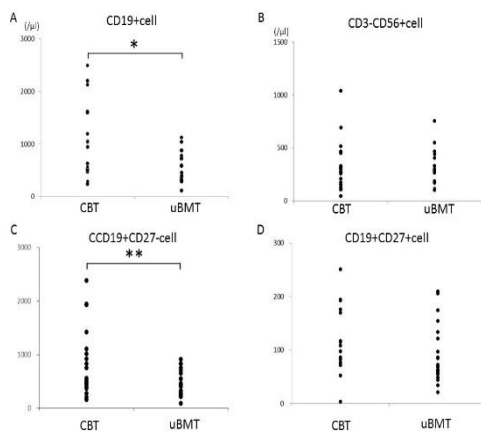


Figure 3.

Figure 3. Differences in B cell recovery between CBT and uBMT group. Absolute cell count of CD19+ cell (A), NK cell (B), naïve B cell (C), and memory B cell (D) of peripheral blood were compared between CBT and uBMT group. * indicates differences that are statistically significant ($p < 0.05$). ** indicates $p = 0.05$.

Complications after allo-HCT including chronic GVHD in CBT and uBMT group

Complications after allo-HCT have been summarized in Table 2. Acute GVHD (grade II-IV) was observed in 7 and 6 patients in CBT and uBMT groups, respectively. CMV antigenemia was more frequently detected in CBT group but cleared in all of cases within 6 months after allo-HCT. In addition, bacterial infections such as sepsis and febrile neutropenia were more frequently complicated in CBT group, as

described previously^{17, 18}. Chronic GVHD was observed in 7 of 21 patients in CBT group and 8 of 20 patients in uBMT group. Three of 7 patients in CBT group and 5 of 8 patients in uBMT group were diagnosed as extended type of chronic GVHD. Continuation of immunosuppressive therapy has been required in 2 and 3 patients in CBT and uBMT group, respectively. We have found no clear difference in the frequency and the severity of chronic GVHD between two groups in our institute, although the incidence of chronic GVHD has been reported to be higher in uBMT rather than CBT patients⁷.

To investigate whether the differences of immune reconstitution would exist between CBT and uBMT group in relation to the development of chronic GVHD, we further divided the patients into 4 groups according to the presence or absence of chronic GVHD as following: the patients with cGVHD in CBT group ($n = 7$), without cGVHD in CBT group ($n = 14$), without cGVHD in uBMT group ($n = 12$), with cGVHD in uBMT group ($n = 8$).

Immune reconstitution in T and B cells in relation to chronic GVHD

We examined T cell recovery between 4 groups described above. There was no significant difference in CD4+ and CD8+ cell number and CD4/CD8 ratio between 4 groups (data not shown). As shown in Figure 4A and B, absolute cell number in peripheral blood of naïve CD4+ but not naïve CD8+ T cells was significantly lower in the patients with cGVHD in uBMT group. Absolute cell number of regulatory T cell was lower in the patients with cGVHD in uBMT group without statistical significance ($p = 0.08$, Figure 4C).

In B cells, absolute cell number of CD19+ cell in peripheral blood was higher in the patients with cGVHD in CBT group, but conversely lower in the patients with cGVHD in uBMT group, as shown in Figure 4D.

These results suggest that there exist some differences in immune reconstitution of both T and B cells in relation to the development of chronic GVHD between CBT and uBMT group.

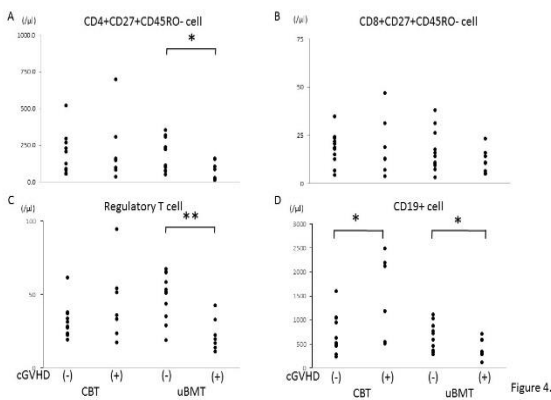


Figure 4. Comparison of immune recovery in T and B cells between the patients with and without chronic GVHD

Absolute cell count of naïve CD4+ cell (A), naïve CD8+ cell (B), regulatory T cell (C), and CD19+ cell (D) of peripheral blood were compared between the patients with and without chronic GVHD in CBT and uBMT group. * indicates differences that are statistically significant ($p < 0.05$). ** indicates $p = 0.08$.

B cell subpopulations in relation to chronic GVHD among CBT and uBMT group

To more precisely investigate the differences in B cell reconstitution between two groups in relation to chronic GVHD, we next examined the subpopulation of B cells.

Both ratio and absolute cell number of naïve B cell, which express CD19 but not CD27, were significantly higher in the patients with than without cGVHD in CBT group (Figure 5A and D). By contrast, absolute cell number but not ratio of naïve B cell was significantly lower in the patients with than without cGVHD in uBMT group (Figure 5A and D). Concerning memory B cells, ratio but not absolute cell number of CD19+CD27+ cell in peripheral blood was significantly lower in the patients with than without cGVHD in CBT group, while there was no significant difference in memory B cells between the patients with and without chronic GVHD in uBMT group

(Figure 5B and E).

Regulatory B cells, which are also suggested to be involved in the pathogenesis of chronic GVHD, have been reported to be enriched in the IgM memory and transitional subsets^{11, 19}. Therefore, we next examined the IgM memory B cells, designated as the cells which express CD19, CD27, and IgM on cell surface. As shown in Figure 5C and F, both ratio and absolute cell count were lower in the patients with cGVHD in CBT group, but the differences were not statistically significant. In uBMT group, we observed no significant difference in IgM memory B cells between the patients with and without chronic GVHD.

These results suggest that different immune reconstitution in T and B cells, which would be related to the development of chronic GVHD, could exist between CBT and uBMT patients.

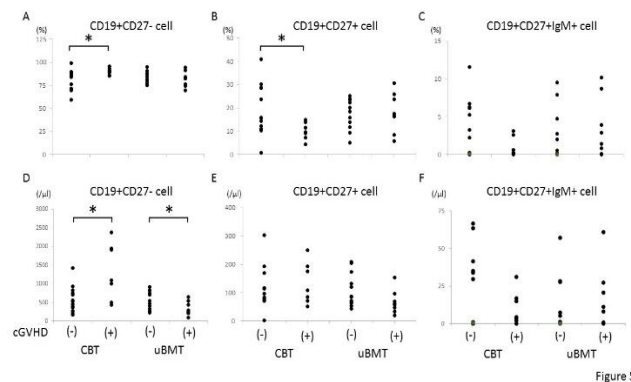


Figure 5. Comparison of immune recovery in B cell subpopulations between the patients with and without chronic GVHD

The ratio of naïve B cell (A), memory B cell (B), and IgM memory B cell (C) of peripheral blood were compared between the patients with and without chronic GVHD in CBT and uBMT group (upper column).

Absolute cell number of naïve B cell (D), memory B cell (E), and IgM memory B cell (F) of peripheral blood were compared between the patients with and without chronic GVHD in CBT and uBMT group (lower column). * indicates differences that are statistically significant ($p < 0.05$).

Table 2. Complications after allo-HCT in the patients who had received cord blood transplantation (CBT) and unrelated bone marrow transplantation (uBMT)

| CBT | | | | | | | | |
|---------|----------------|-----------------|--------|---------------|----------------------|-----------------|-------|------------------|
| Disease | Disease status | Age at allo-HCT | Gender | aGVHD (grade) | infection | CMV antigenemia | cGVHD | Sites of cGVHD |
| AML | NC | 57 | F | I | - | + | L | skin |
| ATL | CR1 | 57 | M | II | sepsis | + | L | skin |
| Ph+ALL | CR2 | 48 | M | I | FN | + | L | mouth |
| DLBCL | CR2 | 51 | F | I | sepsis | + | E | lung |
| CMMoL | NC | 40 | M | II | Tbc | + | E | skin, liver |
| Ph+ALL | CR2 | 44 | M | 0 | FN | + | - | |
| FL | NC | 56 | M | 0 | FN | + | E | lung |
| ALL | CR1 | 32 | F | I | - | + | - | |
| FL | CR3 | 51 | M | 0 | FN | + | L | |
| ALL | CR1 | 41 | M | 0 | sepsis | + | - | |
| AML | CR1 | 45 | M | 0 | - | + | - | |
| AML | CR2 | 45 | F | III | sepsis | - | - | |
| Ph+ALL | CR1 | 39 | F | II | sepsis | + | - | |
| AML | CR2 | 44 | F | II | sepsis | + | - | |
| PTCL | NC | 33 | M | II | sepsis | + | - | |
| AML | CR1 | 52 | F | 0 | FN | + | - | |
| AML | CR1 | 38 | F | II | - | + | - | |
| CML | CP1 | 24 | M | 0 | FN | + | - | |
| ATL | CR1 | 41 | F | 0 | FN | + | - | |
| MDS | RA | 62 | M | I | hemorrhagic cystitis | + | - | |
| Ph+ALL | CR1 | 63 | M | I | - | + | - | |
| uBMT | | | | | | | | |
| Disease | Disease status | Age at allo-HCT | Gender | aGVHD (grade) | infection | CMV antigenemia | cGVHD | Sites of cGVHD |
| AML | CR1 | 25 | F | 0 | - | + | - | |
| MDS | RAEB2 | 54 | M | I | - | + | L | mouth |
| AML | CR1 | 34 | M | II | FN | + | E | lung |
| ALCL | CR2 | 56 | M | II | sepsis | + | E | mouth, eye |
| AML | CR2 | 48 | F | 0 | sepsis | - | - | |
| MM | sCR | 39 | F | I | FN | - | - | |
| DLBCL | CR2 | 44 | F | 0 | - | - | - | |
| FL | CR2 | 46 | F | I | pneumonia | - | E | eye, lung |
| MDS | RAEB2 | 26 | M | 0 | FN | - | - | |
| AML | CR1 | 26 | F | I | - | + | - | |
| MDS | RAEB2 | 38 | M | 0 | FN | - | - | |
| AML | CR1 | 38 | F | I | - | + | - | |
| PNH/AA | | 39 | F | 0 | pneumonia | + | - | |
| AML | CR1 | 49 | M | I | - | + | - | |
| MDS | RAEB2 | 29 | M | III | hemorrhagic cystitis | + | L | skin |
| CML | BC | 35 | F | I | FN | + | E | eye, skin, lung |
| NHL | CR2 | 32 | M | I | FN | + | - | |
| CML | CR2 | 38 | M | III | - | + | E | skin, mouth, eye |
| AML | CR2 | 20 | F | I | - | - | L | skin, eye |
| DLBCL | NC | 48 | F | III | FN | + | - | |

Allo-HCT; allogeneic hematopoietic cell transplantation, CMV; cytomegalovirus, GVHD; graft-versus-host disease, AML; acute myelogenous leukemia, ATLL; adult T cell leukemia/lymphoma, Ph+ALL; Philadelphia chromosome positive acute lymphoblastic leukemia, DLBCL; diffuse large B cell lymphoma, CMMoL; chronic myelomonocytic leukemia, FL; follicular lymphoma, ALL; acute lymphoblastic leukemia, PTCL; peripheral T cell lymphoma, CML; chronic myelogenous leukemia, MDS; myelodysplastic syndrome, ALCL; anaplastic large cell lymphoma, MM; multiple myeloma, PNH/AA; paroxysmal nocturnal hematuria/aplastic anemia, NHL; non-Hodgkin lymphoma, NC; non-complete remission, CR1; first complete remission, CR2; second complete remission, CR3; third complete remission, RA; refractory anemia, RAEB; refractory anemia with excess blast, sCR; stringent complete remission, BC; blastic crisis, CB; cord blood, uBMT; unrelated bone marrow transplantation, FN; febrile neutropenia, Tbc; tuberculosis, L; limited type, E; extensive type.

DISCUSSION

Recent advances of allo-HCT improve the prognosis of the patients with hematopoietic malignancies and result in the long-term survivors, while there have appeared several problems such as chronic GVHD in long-survivors. Development of chronic GVHD results in the decrease of quality of life and increase non-relapse mortality in long-survivors³⁻⁵. Recently, the pathogenesis of chronic GVHD has been elucidated^{8,9}. In the development of chronic GVHD, effector cells as well as regulatory cells have been reported to be involved^{10,11,19}. Several reports have shown the differences in immune recovery between the patients transplanted with cord blood and peripheral blood stem cells from HLA-matched sibling or unrelated donor¹²⁻¹⁴. However, observation period for immune recovery were up to 2 years in these reports. It has been elucidated whether the differences of immune reconstitution between CBT and uBMT patients who survive more than 2 years would exist and relate to the development of chronic GVHD, although the incidence and the severity of chronic GVHD has been reported to be lower in CBT than uBMT patients among Japanese patients⁷.

In this study, we investigated the immunologic reconstitution by flowcytometric analysis in the patients who received CBT or uBMT, although the duration from allo-HCT to investigating time were varied in each patient. We observed several differences in both T- and B-cell lineages between the patients who received with between CBT and uBMT. Absolute cell number of CD8+ T cell was significantly higher in uBMT group, while no difference was observed in CD4+ T cell. As a result, the ratio of CD4/CD8 was significantly lower in uBMT group (Figure 2). In respect to B cells, absolute cell number of B cell was significantly lower in uBMT group (Figure 3A). In addition, absolute cell number of naïve B cell was lower in uBMT group but not significantly (Figure 3C, $p=0.05$). These results suggest that different immune reconstitution between CBT and uBMT patients could exist even more than 2 years after allo-HCT.

Nine of 21 patients in CBT group and 12 of 20 patients in uBMT group had received MAC as conditioning regimen. The difference of conditioning regimen (MAC versus RIC) would possibly affect the

immune reconstitution in the patients who survive more than 2 years after allo-HCT. However, Wike et al. investigated the effect of conditioning regimen on marrow damage and hematopoietic recovery in adult leukemia patients. They reported that MAC regimen including TBI leads to prolonged reduction in marrow cellularity, but does not show additional histological long-term injury and differences in immunologic recovery²⁰. In addition, Geyer et al. also show that immune cell subset recovery, immunoglobulin reconstitution, and the incidence of opportunistic infections did not differ significantly between MAC and RIC regimen in pediatric CBT cohort²¹. These results can suggest that differences of conditioning regimen would not affect immune reconstitution in our investigation.

Regarding the relation to the occurrence of chronic GVHD, several differences have found between the patients with and without chronic GVHD in both CBT and uBMT group.

In respect to T cells, absolute cell number of naïve CD4+ T cell was significantly lower in the patients with chronic GVHD in uBMT group (Figure 4A), while no significant difference was observed in naïve CD8+ T cells and regulatory T cells (Figure 4B and C). Although regulatory T cells have been reported to be involved in the pathogenesis in chronic GVHD¹⁰, we have no significant difference between the patients with and without chronic GVHD in both groups. Although it has been observed that absolute cell number of regulatory T cell was lower in the patients with chronic GVHD in uBMT group, the difference was not statistically significant ($p=0.08$) (Figure 4C). Immune reconstitution of T cells may be different between CBT and uBMT patients even in long-survivors and might be related to the pathogenesis of chronic GVHD at least in uBMT patients.

Concerning B cells, significant differences of absolute cell number of CD19+ B cell between the patients with and without chronic GVHD were observed in both CBT and uBMT group (Figure 4D). In addition, absolute cell number of naïve B cell significantly differed between the patients with and without chronic GVHD in both groups. Absolute cell number of naïve B cell was significantly higher in the patients with chronic GVHD in CBT group, while significantly lower in uBMT group (Figure 5D). As we could not

examine regulatory B cells in this study, we compared IgM memory B cells, which have been reported to contain regulatory B cells¹¹, between CBT and uBMT patients in relation to the development of chronic GVHD. However, there was no significant difference between the patients with and without chronic GVHD in both groups, although IgM memory B cells were lower in the patients with chronic GVHD in CBT group but not statistically significant. However, our results suggest that immune reconstitutions of B cells play important roles in the pathogenesis of chronic GVHD in both CBT and uBMT patients, being consistent to previous reports^{22, 23}.

Taken together, our results suggest that the differences in immune reconstitution of T and B cells may exist between CBT and uBMT patients even in long-term survivors and might be related to the differences in the development of chronic GVHD between CBT and uBMT patients.

Treatment for chronic GVHD has been still difficult and not satisfactory, but new strategies for the treatment of chronic GVHD have been recently evolved²⁴⁻²⁶. Low-dose infusion of interleukin-2 to stimulate regulatory T cells has been reported to improve symptoms of chronic GVHD²⁷. Infusion of rituximab targeting for B cells has also been reported to be effective to chronic GVHD^{26, 28}. Investigating the immunological differences in the development of chronic GVHD between CBT and uBMT would provide the understanding of the pathogenesis of chronic GVHD and the clue to introduce more effective treatment modalities for chronic GVHD.

In this study, there is no significant difference in the observation period from allo-HCT between two groups, although age at allo-HCT was older in CBT group. Moreover, in all of patients, GVHD prophylaxis was calcineurine inhibitor and short-term methotrexate. Our results showed that the differences of immune reconstitution may exist between CBT and uBMT patients even in long-term survivors and might be correlated to the development of chronic GVHD, regardless a small number of patients examined and not being longitudinal analysis in our study. Studies in larger scale and more precise examinations of T and B cells would more clearly reveal the differences in the immune reconstitution and the roles in the

pathogenesis of chronic GVHD in CBT and uBMT patients.

Conflict of interest

The authors declare no conflict of interest.

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