はじめに

従来の同種造血幹細胞移植はbusulfan（BSF 16 mg/kg+cyclophosphamide（CPA）120 mg/kgやCPA 120 mg/kg+全身放射線療法（total body irradiation: TBI）12 Gyを代表とする強力な骨髄破壊的前処置が行われてきた。そのため骨髄破壊的前処置治療に伴う移植関連死亡が少なからず生じ，高齢者や臓器障害を有する患者では適応外であった。骨髄破壊的前処置に用いられるTBIは汎用性が低いのみならず，早期の合併症として粘膜障害，間質性肺炎，晩期の合併症として二次発癌，白内障や，特に小児では成長障害などの発生率が高いとされており，移植前処置法としての問題は多い[1,2]。

一方，内服BSF 16 mg/kg+CPA 120 mg/kgによる前処置法はBSFが内服であるため，肝代謝や年齢，生体内利用率，疾患の状態などの影響を受け，BSFの血中濃度に個体差が生じることが問題になる。BSFの毒性はその血中濃度に依存するとされており，高齢者や病期の進行した患者ではBSFの血中濃度が高くなる傾向があるため，移植後の合併症の発生率を高める原因となる[3-5]。第2寛解期，第2慢性期以降の進行期白血病での内服BSF 16 mg/kg+CPA 120 mg/kgによる移植前処置では移植関連死亡が62%にも及ぶという報告もある[6]。

静注BSF（BUSULFEX®）はBSFの点滴製剤であり，経静脈投与のため肝代謝などの影響を受けず，内服に比べ血中濃度のばらつきが少なく，過量投与による臓器障害や，逆に低濃度が原因による移植片の拒絶，疾患の病勢コントロールが不良になる可能性が低いことが報告されている[7]。

また近年，移植前処置の強度を弱くして副作用を軽減し，高齢者や臓器障害を有する患者にも同種造血幹細胞移植を可能にする目的で骨髄非破壊的移植法が新たな移植法として試みられており，多くの施設で様々な強度，薬剤を組み合わせた移植前処置を用いて臨床研究が行われている[8-13]。しかしながら，骨髄非破壊的前処置では進行期白血病の病勢はコントロールが困難であるという報告もあり，移植前処置の強度を疾患の進行度，年齢や臓器機能に応じ最適化する必要性が生じてきている[14]。

このような背景から，今回われわれは進行期の急性，慢性白血病に対し，移植前処置の強度を至適化する目的で静注BSF 8 mg/kgを用い，TBIの代わりにtotal lymphoid irradiation（TLI）を導入した静注BSF 8 mg/kg+CPA 120 mg/kg+TLI 7.5 Gyによるmodified myeloablative conditioning regimenにて同種造血幹細胞移植を行った。2006年に行われた骨髄非破壊的前処置の定義に関するワークショップでは，内服BSF 9 mg/kg未満を骨髄非破壊的前処置と定義している[15]。本研究で用いた移植前処置はBSFが内服量に換算すると約10 mg/kgに相当する。よって，従来の骨髄破壊的前処置よりは強度は弱いが骨髄非破壊的前処置には当てはまらない強度であり，modified myeloablative conditioning regimenと定義した。

I. 対象および方法

1. 対象症例

対象は2005年1月～2008年5月までに登録を行った進行期白血病，計9例。年齢の中央値は30（18～59）歳。男性6例，女性3例。疾患の内訳は骨髄異形性症候群（MDS）から転化した急性骨髄性白血病（AML）4例［第1寛解期1例，非寛解期3例］，急性リンパ性白血病（ALL）第2寛解期2例，慢性骨髄性白血病（CML）3例［第2慢性期1例，移行期2例］（Table 1）。本研究は2004年2月27日付で大阪市立大学大学院医学研究科の倫理委員会にて承認された。

2. 方　法

移植前処置は，静注BSF 1.6 mg/kg（day－9～－5）+CPA 60 mg/kg（day－4，－3）+TLI 7.5 Gyを用いた。TLIはday－2，－1に2.5 Gy×3 fractionで照射を行った。

造血幹細胞源は血縁骨髄2例，血縁末梢血幹細胞1例，非血縁骨髄6例で，HLA適合度は6例で完全一致，血縁間骨髄移植例1例でHLA-A血清型1座不一致，非血縁間骨髄移植例2例でHLA-DRB1遺伝子座1座不一致であった（Table 1）。血液型は9例中5例で血液型ミスマッチドナーからの移植であった。

急性graft-versus-host disease（GVHD）予防はcyclosporin+短期methotrexate療法で行った。短期methotrexate療法は8例でday 1: 10 mg/m^2，day 3: 7 mg/m^2，day 5: 7 mg/m^2，血縁間骨髄移植の1症例でday 1: 15 mg/m^2，day 3: 10 mg/m^2，day 5: 10 mg/m^{2}で行った。cyclosporin投与は3 mg/kgの1日2分割，3時間点滴静脈投与にて開始し，トラフレベルは150～250 ng/dLを目標として調整を行った。

真菌予防にはフルコナゾールの内服を免疫抑制剤中止まで継続投与した。サイトメガロウイルスantigenemiaは生着後，少なくとも週1回モニターし，5～10陽性/50,000でガンシクロビル5 mg/kg投与開始し，ステロイド投与例などは，1陽性/50,000でガンシクロビル5 mg/kg投与開始を考慮した。

*Pneumocystis jiroveci*肺炎予防には，スルファメトキサゾール・トリメトプリムを用いた。スルファメトキサ

Table 1 Patient characteristics and diagnosis

Characteristics	Number (range)
Number of patients	9
Median age, years	30 (18-59)
Males/females	6/3
Stem cell source related BM/related PB/unrelated BM	2/1/6
Infused dose of BM nuclear cell	1.76×10^8/kg (0.74-3.16)
HLA match/1 antigen mismatch/1 allele mismatch	6/1/2
Diagnosis	**Disease status, number**
MDS/AML	CR1, 1; non-CR, 3
ALL	CR2, 2
CML	2CP, 1; AP, 2

BM: bone marrow, PB: peripheral blood stem cell, CR: complete remission, CP: chronic phase, AP: accelerated phase, AML: acute myeloid leukemia, MDS/AML: secondary acute myeloid leukemia from myelodysplastic syndrome, ALL: acute lymphocytic leukemia, CML: chronic myeloid leukemia

ゾール・トリメトプリムは移植前処置開始前から開始し，day−1から生着確認までは投与中止し，生着後に投与を再開した。ヘルペスウイルス感染予防のアシクロビルは生着まで投与した。ただし，ステロイド投与などの強力な免疫抑制療法施行時は，生着後も続行した。

Ⅱ．結　果

骨髄移植8例での輸注総有核細胞数は1.76 (range: 0.74～3.16)×10^8/kgで，血縁末梢血幹細胞移植症例の1例では，4.47×10^6/kgのCD34陽性細胞の輸注を行った。9例中7例でG-CSFの投与を行った。全例で好中球生着を認め，好中球生着までの日数（好中球数が3日間連続で500/μL以上に達した初日を生着日とした）の中央値は16 (range: 13～20) 日，血小板生着までの日数（7日以内に血小板輸血がなく，3点以上の連続した検査日で，血小板数が2×10^4/μLを上回った初日を生着日とした）の中央値は33 (24～46) 日で，1例で血小板2×10^4/μL以上の回復は認めなかった。

また，移植後60日までに5例 (56%)，100日以内に7例 (78%) が完全ドナーT細胞キメリズムを達成した。残りの1例は拒絶，もう1例は原疾患の再発にて完全ドナーT細胞キメリズムに達しなかった。拒絶された1例はその後再移植を行い生着が得られた。

grade 4の非血液毒性は認めなかった (Table 2)。grade 3の粘膜障害（口内炎，咽頭炎）を6例，grade 3の消化管障害（嘔気，下痢）を8例で認めた。day 30までの37.6℃以上の有熱期間は14 (3～21) 日であった。

サイトメガロウイルス抗原血症は9例中4例に認めたが，サイトメガロウイルス病の発症は全例で認めなかった。その他のウイルス感染症はBKウイルス性出血性膀胱炎 (2例)，アデノウイルス性出血性膀胱炎 (1例)，帯状疱疹ヘルペス (1例)，尖圭コンジローマ (1例) の発症をそれぞれ別の患者で認めた。真菌感染症については1例でカンジダ腸炎を発症した。

Table 2

	Grade, No. episodes (% of patients)		
NCI-CTC ver. 3.0	1-2	3	4
Cardiac	1 (11)	0 (0)	0 (0)
Mucositis	0 (0)	7 (67)	0 (0)
Pulmonary	0 (0)	0 (0)	0 (0)
Gastrointestinal	3 (33)	9 (89)	0 (0)
Hepatic	1 (11)	0 (0)	0 (0)
Renal	0 (0)	0 (0)	0 (0)
Hemorrhage	0 (0)	0 (0)	0 (0)

NCI-CTC: National Cancer Institute-Common Toxicity Criteria

急性GVHDは9人中6例 (67%) に出現し，全例でgrade 2であった。grade 2以上の急性GVHDに対する初回治療は1例を除き，methylprednisoloneまたはprednisolone 1 mg/kgで行い，全例，ステロイド投与のみでGVHDはコントロールできた。皮膚の限局した急性GVHDの1症例では，ステロイドの外用剤のみでコントロール可能であった。慢性GVHDは評価可能症例7例全例（限局型慢性GVHD 4例，広範型慢性GVHD 3例）に認められた。

移植後観察期間中央値は813 (248～1,702) 日で，9例中5例 (MDS/AML non-CR: 3例，ALL CR2: 1例，CML 2CP: 1例) で，移植後，原疾患の再発，再燃を認めた。再発，再燃時期の中央値は移植後337 (181～1,569) 日で，移植後100日以内の再発例はなかった。移植全観察期間

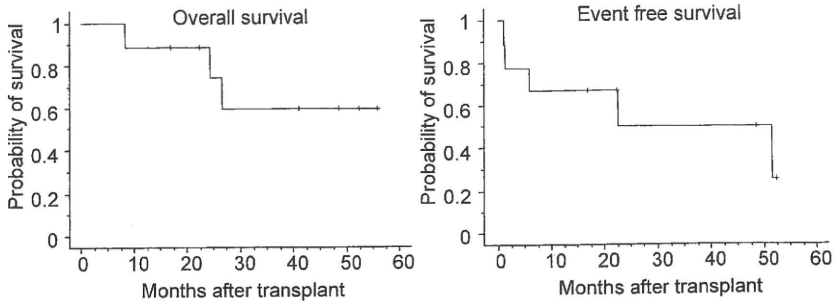

Fig. 1 Kaplan-Meier product estimates of overall survival and event-free survival

を通じて移植関連死亡は認めなかった。2年の全生存率は88.9%，無病生存率は50.0%，3年の全生存率は59.3%，無病生存率は50.0%であった（Fig. 1）。

Ⅲ. 考　察

今回のわれわれの用いた移植前処置による同種造血幹細胞移植では全観察期間で移植関連死亡を認めておらず，2年の全生存率は88.9%，無病生存率は50.0%と比較的良好な結果を得た。静注BSF 8 mg/kgは内服のBSFのおよそ10 mg/kgに相当し，従来の内服BSF 16 mg/kgに比べ約37.5%投与量を減量したことや，静脈内投与を用いたことでBSFの血中濃度の安定化が得られたことが，移植関連死亡の低下につながった可能性があると推測している。

BSFの量を減量することで，特に非血縁間骨髄移植においては，生着不全や拒絶の危険が高まる可能性があった。今回の研究では，生着を担保する目的でBSFの量を減量した代わりにTLIによる放射線治療を加えた。TLIはTBIに比して照射範囲が狭く，臓器毒性が低い可能性が考えられる。今回の研究ではgrade 4の非血液毒性は認めなかったが，9例中8例の患者においてgrade 3の粘膜障害（口内炎，咽頭炎）を認めた。しかしながら，移植関連死亡につながった症例はなかった。神田らによる日本骨髄バンクのデータの後方視的な解析では，BSF+CPAにTLIを加えた移植前処置は生着不全や再発を減らすが，むしろ有意に非再発死亡を増やすことを報告している[16]。しかしながら，BSFの量が多く，投与方法も本研究と異なるため，われわれの研究結果と単純比較はできないと考えられる。

また，ミシガンのグループは，TBIを移植前処置に用いた場合は組織障害が強く，GVHDのリスクが高まる可能性があることからTBIの代わりにTLIを併用した移植前処置を用いており，GVHDの頻度が少ないことを報告している[17]。今回のわれわれの結果でも，grade 2のGVHDは67%と頻度は高かったが，多くが非血縁の骨髄移植にもかかわらずgrade 3，4の急性GVHDは認めなかった。口内炎や咽頭炎などの粘膜障害は比較的高頻度にみられたが，腸管毒性などはTLIはTBIに比して低い可能性があり，TLIを併用した移植前処置が重度のGVHDの発症頻度の低下につながっている可能性もある。

2006年に行われた骨髄非破壊的前処置の定義に関するワークショップの基準[15]に当てはめると，本研究の移植前処置も骨髄破壊的前処置に相当することになるが，BSFの量を減量したことで疾患の早期再発が懸念された。実際，Shimoniらの報告では，fludarabine 150～160 mg/kg＋静注BSF 12.8 mg/kgを用いた骨髄破壊的前処置に比べて，fludarabine 150～160 mg/kg＋静注BSF 6.4 mg/kgによる骨髄非破壊的前処置では進行期白血病の病勢はコントロールが困難であることが示されている[14]。しかしながら，われわれの結果では進行期白血病を対象としたため，再発率は全体では9例中5例（56%）と高かったものの再発時期の中央値は移植後337日で，100日以内の再発例はなかった。移植後期の再発については，移植前処置の効果だけでなくgraft-versus-leukemia（GVL）効果の出現の有無も大きく影響していると考えられ，再発した症例では移植前処置の強度が不足していることよりも，移植後の十分なGVL効果が発揮されなかったためと推測している。

今回の研究では通常の骨髄破壊的移植と同等の治療関連毒性の出現を認めたものの，移植後関連死亡に至った症例はなく，結果として比較的良好な成績を得ることができた。過剰な移植前処置強度は移植関連死亡の増加につながる可能性がある。一方，強度不足は特に進行期白血病では移植後の早期再発を増やす可能性が懸念される。

今回われわれが用いた移植前処置では早期再発例が少なく，現時点で移植関連死亡は認めていないことから，従来の骨髄破壊的前処置の強度を調節することによって，移植関連死亡を減少させると同時に，病勢をコント

ロールし得る移植前処置の適正強度が存在する可能性が示唆された。しかしながら，今回の研究は症例数が少ないので断定的なことはいえない。同種造血幹細胞移植の前処置の至適化には今後，前向きの大規模な臨床試験が必要と考えられる。

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Serum Cytokine Profiles at the Onset of Severe, Diffuse Alveolar Hemorrhage Complicating Allogeneic Hematopoietic Stem Cell Transplantation, Treated Successfully with Pulse Intravenous Cyclophosphamide

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Key Words
Adult T-cell leukemia/lymphoma · Allogeneic hematopoietic stem cell transplantation · Diffuse alveolar hemorrhage · Pulse cyclophosphamide treatment

Abstract
A 59-year-old man with lymphoma-type adult T-cell leukemia/lymphoma was admitted to hospital for treatment of a skin relapse on day 398 after allogeneic hematopoietic stem cell transplantation (allo-HSCT). To induce a graft-versus-adult T-cell leukemia/lymphoma effect, we discontinued methylprednisolone and tacrolimus. About a month after the discontinuation, he developed grade II acute graft-versus-host disease (GVHD) with a high fever. Soon after the development of GVHD, all the skin lesions regressed in size and finally vanished. However, he developed diffuse alveolar hemorrhage (DAH), which was resistant to high-dose corticosteroid therapy. He was intubated for respiratory insufficiency on day 451. Cyclophosphamide pulse therapy was administered at a dose of 1 g per day for 2 days and his oxygen saturation then improved, and ventilatory support was released on day 465. On analysis of cytokine profiles at the onset of DAH, we found elevated serum levels of T-helper 2 cytokines as well as T-helper 1 cytokines, suggesting that both T-helper 1 and T-helper 2 cytokines might play a role in the occurrence of DAH following allo-HSCT. Pulse cyclophosphamide treatment might be very effective in suppressing the exaggerated allogeneic immune response in DAH.

Introduction

Diffuse alveolar hemorrhage (DAH) is a noninfectious pulmonary complication that occurs in approximately 3–7% of allogeneic hematopoietic stem cell transplantation (allo-HSCT) recipients, usually in the early post-transplant phase [1, 2]. Although the etiology of DAH is still unclear, graft-versus-host disease (GVHD), release of inflammatory cytokines and/or lung tissue injury have been considered to play a role in the pathogenesis of DAH

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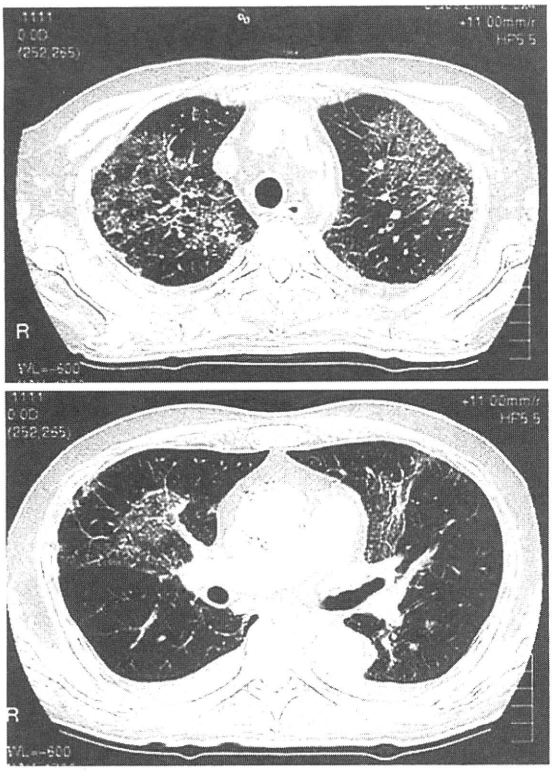

Fig. 1. A chest CT scan on day 442 after allo-HSCT. The chest CT shows multifocal, segmental, ground-glass opacities in the upper and mid-lung fields bilaterally, with bilateral pleural effusions.

after allo-HSCT [1]. Systemic corticosteroid therapy, plasma exchange and/or plasmapheresis have been tried in the treatment of DAH in allo-HSCT recipients [1]. Even with these interventions, DAH following allo-HSCT still carries a very high mortality rate of 48–100% [1, 3–5].

Pulse cyclophosphamide treatment has been used for various conditions such as autoimmune diseases and vasculitis [6–9] and is also useful in most cases of pulmonary alveolar hemorrhage in the non-HSCT setting [10].

Here, we describe successful pulse cyclophosphamide treatment for severe DAH refractory to corticosteroid therapy after allo-HSCT, as well as the cytokine profile in the peripheral blood at the onset of DAH.

Case Report

In October 2005, a 59-year-old man with lymphoma-type adult T-cell leukemia/lymphoma (ATL/L) achieved his first complete remission after 3 courses of the Lymphoma Study Group-15 regimen [11]. Subsequently, he received a marrow graft from a serologically human leukocyte antigen (HLA)-A, HLA-B and HLA-DR compatible but HLA-DRB1 allele mismatched, unrelated male donor, following conditioning with fludarabine 30 mg/m^2 once daily on days –9 to –4 and intravenous busulfan 0.8 mg/m^2 twice daily on days –9 to –5. The number of nucleated cells was 5.01 × 10^8/kg recipient weight. GVHD prophylaxis consisted of cyclosporine A 3 mg/kg alone from day –3. At day 7 after allo-HSCT, he developed skin rash, and grade II acute GVHD was diagnosed based on histologic findings from the skin. Acute GVHD resolved immediately after initiating intravenous methylprednisolone (mPSL) and steroid was subsequently tapered gradually.

On day 398 after allo-HSCT, a 5 × 10 mm erythematous nodule appeared in the right axilla, and multiple, slightly raised, small erythematous nodules appeared on the abdomen. He was admitted to hospital for a suspected skin relapse. We performed a skin biopsy and southern blot analysis using the biopsy sample and diagnosed ATL/L relapse. To induce a graft-versus-ATL/L effect, we discontinued oral mPSL (a total of 2 mg) and tacrolimus. About a month after discontinuation of the immunosuppressants, grade II acute GVHD flared up, with a high fever. Soon after the development of acute GVHD, all the skin lesions slowly decreased in size and finally vanished. Around the same time, hypoxemia occurred, and thus, we performed chest radiography and a computed tomography (CT) scan on day 442. The chest radiography showed bilateral, diffuse consolidation in the upper and mid-lung fields, and the chest CT revealed multifocal, segmental, ground-glass opacities in the same regions, with bilateral pleural effusions (fig. 1). There was no evidence of cardiac failure clinically. Broad-spectrum antibiotics, an antifungal agent, ganciclovir and intravenous prednisolone (1 mg/kg) were initiated. After the administration of prednisolone, skin GVHD gradually disappeared. Despite these therapies, the patient's general condition progressively deteriorated and he started having hemoptysis. Although we added mPSL pulse therapy at 1 g per day for 3 days, and continuous intravenous infusion of tacrolimus and sivelestat sodium hydrate, he was intubated for respiratory insufficiency on day 451. Copious amounts of bleeding occurred from the respiratory tract and filled the endotracheal tube. However, the platelet count in the peripheral blood and blood coagulation tests were normal (platelet count 221 × 10^9/l; prothrombin time 11.6 s; partial thromboplastin time 25.1 s; international normalized ratio 0.89; fibrinogen level 317 mg/dl; and fibrinogen degradation product level 4.3 μg/ml). The following day, bronchoscopic examination was performed and bronchoalveolar lavage (BAL) fluid showed progressively bloodier return from more than 3 subsegmental bronchi. Semiquantitative counting by light microscopy showed that the BAL sample predominantly contained macrophages and a moderate number of neutrophils. Pathological evaluation showed that 26.2% of the macrophages were hemosiderin laden. Gram and acid-fast stains, cultures for bacteria and acid-fast bacilli, fungal cultures, as well as antigen detection of *Aspergillus* galactomannan, *Candida* and *Cryptococcus* were performed on the BAL specimen. Viral PCR studies were also carried out on the BAL specimen to detect viruses including adenovirus, respi-

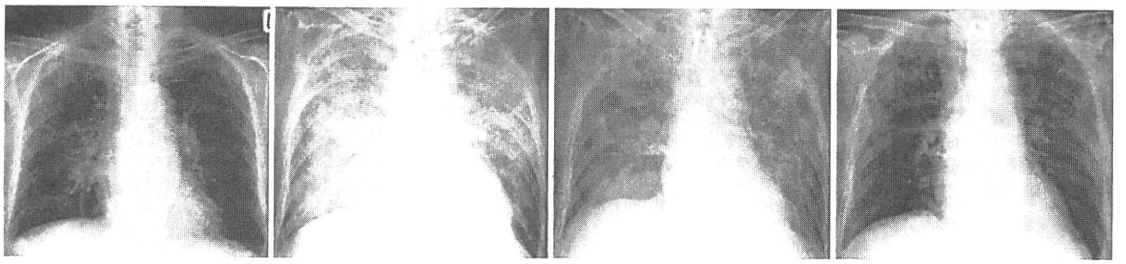

Fig. 2. The chest X-rays on days 442, 452, 459 and 467 after allo-HSCT. On day 451, the patient was intubated for mechanical ventilation. On day 452, intravenous cyclophosphamide pulse therapy was given, at a total dose of 1 g per day for 2 consecutive days.

ratory syncytial virus, parainfluenza virus type III, cytomegalovirus, herpes simplex virus, and varicella zoster virus. Moreover, cytologic studies were performed using Papanicolaou, Grocott, and periodic acid-Schiff stains. However, these revealed no evidence of any infection or ATL/L involvement. Based on these findings we diagnosed DAH [1]. In addition, blood culture, serum β-D-glucan level and *Aspergillus* galactomannan in blood all remained negative throughout the follow-up period. On day 452, we initiated intravenous cyclophosphamide pulse therapy at a total of 1 g per day for 2 consecutive days. Thereafter, his oxygen saturation and chest radiography gradually improved (fig. 2). He was extubated on day 465. At day 598 after allo-HSCT, he was discharged without any evidence of recurrent ATL/L or GVHD.

Cytokine Profile at the Onset of DAH

The Bio-Plex Pro Cytokine Assay® system combines the principle of a sandwich immunoassay with Luminex fluorescent bead-based technology. This system allows individual and multiplex analysis of up to 100 different analytes in a single microtiter well [12].

Sera were collected and subjected to analysis of cytokines by the cytokine assay system, according to the manufacturer's instructions. Cytokines included interleukin (IL)-1β, IL-1rα, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, eotaxin, fibroblast growth factor basic, granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, interferon (IFN)-γ, IFN-γ-inducible protein 10, monocyte chemoattractant 1, macrophage inflammatory proteins 1α and 1β, platelet-derived growth factor-ββ, regulated on activation, normal T cell expressed and secreted, tumor necrosis factor-α and vascular endothelial growth factor. Serum samples from peripheral blood were collected from the patient before allo-HSCT and at the onset of DAH. The serum was separated by centrifuging the blood at 3,500 rpm for 10 min and the serum samples were then stored at −80°C until they were analyzed.

As shown in table 1, the development of DAH was associated with a greater than 2-fold increase in peripheral blood concentrations of several inflammatory and anti-inflammatory mediators, including both T-helper 1 (Th1) cytokines (tumor necrosis factor-α, IFN-γ, IL-2) and Th2 cytokines (IL-9, IL-10 and IL-15), when compared with values obtained before allo-HSCT.

Discussion

DAH occurs frequently in the first 30 days after HSCT [1, 2]. The reported mortality rate is extremely high [1, 3–5]. Furthermore, in our case, DAH occurred in the late posttransplant phase. Late-onset DAH was associated with a significantly poorer prognosis than early DAH [3]. Although there is no well-established treatment for DAH, several recent case reports showed the potential effectiveness of recombinant factor VIIa [13, 14], aminocaproic acid [15] and extracorporeal life support [16]. We administered high-dose cyclophosphamide for DAH refractory to corticosteroid therapy. Cyclophosphamide targets proliferating alloreactive T cells [17]. Thus, in our case, successful suppression of the exaggerated allogeneic immune response by treatment with high-dose cyclophosphamide possibly led to the cure of DAH.

The mechanism of DAH after allo-HSCT has not been clearly elucidated. In our case, after discontinuation of the immunosuppressants, DAH occurred concurrently

Table 1. Concentrations of cytokines and chemokines before HSCT and with diffuse alveolar hemorrhage

Cytokine or chemokine	Before HSCT pg/ml	Onset pg/ml	Increase ratio
Eotaxin	97.84	119.28	1.22
FGF basic	OOR<	OOR<	-
G-CSF	11.95	17.32	1.45
GM-CSF	OOR<	OOR <	-
IFN-γ	38.67	172.15	4.45
IP-10	1,550.12	7,890.94	5.09
MCP-1	30	55.55	1.85
MIP-1α	4.12	2.43	0.59
MIP-1β	142.27	126.62	0.89
PDGF-ββ	8,620.87	12,249.7	1.42
RANTES	OOR>	OOR>	-
TNF-α	9.95	50.91	5.12
VEGF	133.96	84.56	0.63
IL-1β	1.31	2.01	1.53
IL-1rα	84.63	145.62	1.72
IL-2	OOR<	7.65	2.0<
IL-4	1.73	2.69	1.55
IL-5	0.91	1.6	1.76
IL-6	3.89	95.01	24.42
IL-7	8.48	15.11	1.78
IL-8	7.62	12.42	1.63
IL-9	OOR<	54.89	2.0<
IL-10	1.05	2.81	2.68
IL-12	9.11	14.73	1.62
IL-13	3.16	3.83	1.21
IL-15	OOR<	3.93	2.0<
IL-17	OOR<	OOR<	-

FGF = Fibroblast growth factor; OOR< = out of range below; G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte macrophage colony-stimulating factor; IP-10 = IFN-γ-inducible protein 10; MCP-1 = monocyte chemoattractant 1; MIP = macrophage inflammatory protein; PDGF-ββ = platelet-derived growth factor-ββ; RANTES = regulated on activation, normal T cell expressed and secreted; OOR> = out of range above; TNF-α = tumor necrosis factor-α; VEGF = vascular endothelial growth factor.

with the onset of GVHD, suggesting an association between the allogeneic immune response and DAH. DAH could be considered a subset of idiopathic pneumonia syndrome in which acute pulmonary hemorrhage is present, associated with hemorrhagic alveolitis [18]. Previous reports of idiopathic pneumonia syndrome also showed that Th1 cytokines play a critical role in the occurrence of idiopathic pneumonia syndrome [18–20]. On analysis of cytokine profiles in peripheral blood at the onset of DAH, we found that a wide diversity of cytokines including both Th1 and Th2 were overtly raised at the onset of DAH, although Th1 cytokines predominated compared with Th2 cytokines. Th1 and Th2 cells are characterized by mutually exclusive patterns of cytokine production with different functions and reciprocal differentiation, and Th1 cytokines are generally known to functionally oppose the effects of Th2 cytokines. Although not proven, our results might indicate dysregulation of cytokine balance in allo-HSCT, or interaction between Th1 and Th2 cytokines. Furthermore, in a murine model, it was reported that Th1 and Th2 cytokines target distinct end-organs and work additively in acute GVHD; additionally, deficiency in IFN-γ in donor CD4+ T cells results in augmented differentiation of Th2 and Th17 cells, which preferentially cause damage in lung tissue [21, 22]. Therefore, we postulated that not only Th1 but also Th2 cytokines play an important role in the pathogenesis of DAH after allo-HSCT.

We have shown that pulse cyclophosphamide treatment is very effective for DAH that is refractory to corticosteroid therapy. Cyclophosphamide has a favorable safety profile [23] and, in fact, can be administered safely to prevent acute GVHD, even after allo-HSCT [24–26]. Further prospective study is warranted to confirm the safety and efficacy of pulse cyclophosphamide treatment for DAH after allo-HSCT.

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Transplantation of allogeneic hematopoietic stem cells for adult T-cell leukemia: a nationwide retrospective study

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Transplantation of allogeneic hematopoietic stem cells for adult T-cell leukemia: a nationwide retrospective study

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Allogeneic hematopoietic stem cell transplantation (HSCT) is increasingly used as a curative option for adult T-cell leukemia (ATL), an intractable mature T-cell neoplasm causally linked with human T-cell leukemia virus type I (HTLV-I). We compared outcomes of 386 patients with ATL who underwent allogeneic HSCT using different graft sources: 154 received human leukocyte antigen (HLA)–matched related marrow or peripheral blood; 43 received HLA-mismatched related marrow or peripheral blood; 99 received unrelated marrow; 90 received single unit unrelated cord blood. After a median follow-up of 41 months (range, 1.5-102), 3-year overall survival for entire cohort was 33% (95% confidence interval, 28%-38%). Multivariable analysis revealed 4 recipient factors significantly associated with lower survival rates: older age (> 50 years), male sex, status other than complete remission, and use of unrelated cord blood compared with use of HLA-matched related grafts. Treatment-related mortality rate was higher among patients given cord blood transplants; disease-associated mortality was higher among male recipients or those given transplants not in remission. Among patients who received related transplants, donor HTLV-I seropositivity adversely affected disease-associated mortality. In conclusion, allogeneic HSCT using currently available graft source is an effective treatment in selected patients with ATL, although greater effort is warranted to reduce treatment-related mortality. (*Blood*. 2010;116(8):1369-1376)

Introduction

Adult T-cell leukemia (ATL) is a mature T-cell neoplasm developing in a minority of persons infected with human T-cell leukemia virus type I (HTLV-I), the first retrovirus isolated from a human malignant disease.[1-4] HTLV-I is estimated to infect 10 to 20 million people worldwide and is endemic in some areas of Japan, sub-Saharan Africa, the Caribbean Basin, and South America.[5,6] The area with the highest HTLV-I prevalence is the Kyushu district in southwestern Japan, where more than 10% of the general population is infected and the cumulative incidence of developing ATL among adult virus carriers is estimated at approximately 6.6% for males and 2.1% for females.[7] The onset of ATL after HTLV-I infection appears to require a long latency period because the median age at diagnosis ranges from 40 to 60 years in most endemic regions where mother-to-child viral transmission had been previously common.[4-6]

Clinical manifestation of ATL is heterogeneous and characterized by various degrees of lymphadenopathy, abnormal lymphocytosis, hepatosplenomegaly, skin lesions, and hypercalcemia, dividing the disease into 4 subtypes: acute, lymphomatous, chronic, and smoldering.[8] Patients with acute or lymphomatous type had extremely poor prognosis, mainly because of resistance to a variety of cytotoxic agents and susceptibility to opportunistic infections. Chronic and smoldering forms have relatively indolent clinical courses but can transform into more aggressive subtypes. During the past 3 decades since the clinical discovery of ATL,[1] the results of conventional cytotoxic chemotherapy remain dismal because of low response rates and lack of long-term efficacy. The

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median survival time that followed the best clinical results to date is approximately 13 months[9,10]; complete response can only be achieved in 25%-40% of treated cases and most of them eventually relapsed with the median progression-free survival time of 5 to 7 months, whereas available treatment options are extremely limited in those who failed initial chemotherapy.[11-14]

Although the early experience of ablative chemoradiotherapy with autologous hematopoietic stem cell rescue for ATL resulted in a high incidence of relapse and fatal toxicities,[15] allogeneic hematopoietic stem cell transplantation (HSCT) has been explored as a promising alternative that can provide long-term remission in a proportion of patients with ATL.[16-19] Although the mechanisms by which allografting can eradicate HTLV-I–infected neoplastic T cells are not fully elucidated, several reports have suggested the role of graft-versus-HTLV-I or graft-versus-ATL effects.[20-23] Over the past decade, improved access to alternative stem cell sources and the development of less toxic conditioning regimens have led to a rapid increase in the number of cases of ATL treated with allogeneic HSCT, albeit without consistent efficacy.[24-30] Therefore, we conducted a nationwide retrospective cohort study to identify pretransplantation factors that affect survival after allografting for ATL, with special emphasis on the effect of graft source: we compared the outcomes of human leukocyte antigen (HLA)–mismatched related bone marrow or peripheral blood transplantation, unrelated bone marrow transplantation, and unrelated cord blood transplantation with those of HLA-matched related bone marrow or peripheral blood transplantation as treatment for ATL. We also evaluated the effect of donor HTLV-I serostatus on outcomes among patients who received transplants from related donors.

Methods

Collection of data

Data on 417 patients with acute or lymphomatous type ATL who had received T-cell–replete allogeneic bone marrow, peripheral blood, or cord blood transplantation between January 1, 1996, and December 31, 2005, were collected through the 3 largest hematopoietic cell transplant registries in our country: the Japan Society for Hematopoietic Cell Transplantation (JSHCT), the Japan Marrow Donor Program (JMDP), and the Japan Cord Blood Bank Network (JCBBN). The patients were included from 102 transplant centers; the data were updated as of December 2008. To evaluate the effect of HTLV-I infection in donors on transplantation outcomes, additional questionnaires were sent to 77 centers in January 2010 to retrieve data on donor HTLV-I serostatus in 217 related transplants registered with the JSHCT. Our analysis included patients for whom there was data on age at transplantation, sex, donor type, stem cell source, and agents used in the conditioning regimen and graft-versus-host disease (GVHD) prophylaxis. Twenty-two patients who missed any of these data, and 8 patients who had a history of prior autologous or allogeneic stem cell transplantation were excluded from the analysis. One patient who had received an ex vivo T-cell–depleted graft was also excluded. Two independent physicians reviewed the quality of collected data, and a total of 386 patients (209 males and 177 females), with a median age of 51 years (range, 18-79 years), were found to fulfill the inclusion criteria: 197 patients from JSHCT, 99 from JMDP, and 90 from JCBBN. No overlapping cases were identified. Data on engraftment or graft failure were missing in 23 patients. Data on acute GVHD were not available in 53 patients because of early death or missing data.

The JSHCT registry currently includes more than 390 transplant centers variously located in Japan and collects data on transplantation by use of autologous or related stem cell grafts. The JMDP includes more than 190 centers and collects data on unrelated bone marrow transplantation. The JCBBN, a national network of 11 cord blood banks, collects data on unrelated cord blood transplantations reported individually from more than 220 transplant centers to each bank. Participating centers to these registries are requested to report each type of transplantation consecutively and longitudinally. Until 2005, the 3 registries were operated separately from one another; however, a project attempting to unify them has been launched via development of the Transplant Registry Unified Management Program, which enables participating centers to use a shared format for data submission to each registry.[31] All unrelated donor transplants in Japan were facilitated through the JMDP and JCBBN, although peripheral blood donation from unrelated volunteers has not yet been instituted as of March 2010. The study was approved by the data management committees of the JSHCT, JMDP, and JCBBN, as well as by the institutional review boards of Kyoto University, Graduate School of Medicine, where this study was organized.

End points

The primary end point of the study was overall survival, defined as the time from the date of transplantation until date of death from any cause. Patients who remained alive at the time of last follow-up were censored. Reported causes of death were reviewed and categorized into disease-associated or treatment-associated deaths. Disease-associated deaths were defined as deaths from relapse or progression of ATL among patients who survived for at least 30 days after transplantation. Treatment-related deaths were defined as any death other than disease-associated deaths. Neutrophil recovery was considered to have occurred when an absolute neutrophil count exceeded 0.5×10^9/L for 3 consecutive days after transplantation. Primary graft failure was evaluated in patients who survived at least 30 days and was defined as no evidence of neutrophil recovery after transplantation. Acute and chronic GVHD were diagnosed and graded using traditional criteria by the physicians who performed transplantations at each center.[32,33] The incidence of acute GVHD was evaluated in patients who survived for at least 7 days, and that of chronic GVHD was evaluated in patients who survived for at least 100 days.

Statistical analysis

Descriptive statistics were used for summarizing variables related to patient demographics and transplant characteristics. Comparisons among the groups were performed by use of the χ^2 statistic or extended Fisher exact test as appropriate for categorical variables, and the Kruskal-Wallis test for continuous variables. The probability of overall survival was estimated according to the Kaplan-Meier method, and univariable comparisons among the groups were made using the log-rank test. Probabilities of acute and chronic GVHD, treatment-related mortality, and disease-associated mortality were estimated with the use of cumulative incidence curves to accommodate the following competing events[34]: death without GVHD for acute and chronic GVHD, disease-associated death for treatment-related mortality, and treatment-related death for disease-associated mortality. Data on patients who were alive at the time of last follow-up were censored. Cox proportional-hazards regression was used to evaluate variables potentially affecting overall survival, whereas Fine and Gray proportional-hazard model was used to evaluate variables affecting other outcomes.[35] The variables considered were recipient age group ($\leq$ 50 years or $>$ 50 years at transplantation); recipient sex; disease status before transplantation; type of conditioning regimen; type of GVHD prophylaxis; type of graft source; time from diagnosis to transplantation (within 6 months or longer than 6 months); and year of transplantation. Only factors differing in distribution among the graft source groups and factors associated with outcomes by univariable comparison were included in the final models. The effect of donor HTLV-I seropositivity on outcomes after related donor transplantation was also evaluated by univariable and multivariable analysis with the use of data on 156 patients given transplants from siblings or other related family members for whom data on the HTLV-I serostatus were available. Results were expressed as hazard ratios and their 95% confidence interval (CI). All tests were 2-sided, and a P value of less than .05 was considered to indicate statistical significance. All statistical analyses were performed with STATA software (Version 11; Stata Corporation).

Results

Patients

Table 1 shows characteristics of the patients and transplantation procedures. Compared with HLA-matched related bone marrow or

Table 1. Characteristics of allografted patients with ATL

Patient variables	No. of recipients by graft source type (%)				
	HLA-matched related bone marrow or peripheral blood (N = 154)	HLA-mismatched related bone marrow or peripheral blood (N = 43)	Unrelated bone marrow (N = 99)	Unrelated cord blood (N = 90)	*P*
Age range at transplantation, y					.001
30 or younger	4 (3)	1 (2)	2 (2)	1 (1)	
30-40	21 (14)	4 (9)	8 (8)	3 (3)	
40-50	56 (36)	12 (28)	44 (44)	21 (23)	
50-60	57 (37)	22 (51)	43 (43)	47 (52)	
Older than 60	16 (10)	4 (9)	2 (2)	18 (20)	
Sex					.257
Male	76 (49)	21 (49)	60 (61)	52 (58)	
Female	78 (51)	22 (51)	39 (39)	38 (42)	
Disease status					.001
Complete remission	50 (32)	7 (16)	35 (35)	26 (29)	
Not in complete remission	102 (66)	35 (81)	52 (53)	57 (63)	
Unknown	2 (1)	1 (2)	12 (12)	7 (8)	
Conditioning regimen					< .001
CY-TBI or BU-CY	51 (33)	6 (14)	43 (43)	14 (16)	
Purine analog–containing	72 (47)	23 (53)	37 (37)	64 (71)	
Others	31 (20)	14 (33)	19 (19)	12 (13)	
GVHD prophylaxis					< .001
Cyclosporine-based	146 (95)	11 (26)	29 (29)	60 (67)	
Tacrolimus-based	6 (4)	31 (72)	68 (69)	25 (28)	
Others	2 (1)	1 (2)	2 (2)	5 (6)	
Source of stem cells					< .001
Bone marrow	46 (30)	12 (28)	99 (100)	-	
Peripheral blood	106 (69)	31 (72)	-	-	
Bone marrow + peripheral blood	2 (1)	0 (0)	-	-	
Cord blood	-	-	-	90 (100)	
HLA compatibility*					
Matched	154 (100)	-	83 (84)	3 (3)	< .001
One-antigen mismatch		19 (44)	12 (12)	29 (32)	
Two-antigen mismatch		13 (30)	0 (0)	57 (63)	
Three-antigen mismatch		7 (16)	0 (0)	1 (1)	
Uncertain/missing		4 (9)	4 (4)	0 (0)	
Time from diagnosis to transplantation					< .001
6 months or less	92 (60)	26 (60)	22 (22)	49 (54)	
More than 6 months	52 (34)	16 (37)	75 (76)	41 (46)	
Uncertain/missing	10 (6)	1 (2)	2 (2)	0 (0)	
Year of transplantation					< .001
1995-1999	18 (12)	1 (2)	5 (5)	0 (0)	
2000-2002	66 (43)	15 (35)	26 (26)	12 (13)	
2003-2005	70 (45)	27 (63)	68 (69)	78 (87)	
Follow-up of survivors†					
Median mo (range)	40.5 (1.5-102.3)	36.7 (8.8-85.1)	40.2 (16.0-81.2)	48.9 (1.6-73.5)	.847

ATL indicates adult T-cell leukemia; HLA, human leukocyte antigen; GVHD, graft-versus-host disease; CY-TBI, cyclophosphamide with total-body irradiation; BU-CY, busulfan and cyclophosphamide; purine analog–containing, conditioning regimens containing fludarabine, cladribine, or pentostatin; cyclosporine-based, cyclosporine with or without other agents; and tacrolimus-based, tacrolimus with or without other agents.

*HLA compatibility was defined according to the results of serologic or low-resolution molecular typing for HLA-A, HLA-B, and HLA-DR antigens.

†Data are time interval in months.

peripheral blood recipients, HLA-mismatched bone marrow or peripheral blood recipients were more likely to receive tacrolimus for GVHD prophylaxis; unrelated bone marrow recipients took a longer time from diagnosis to transplantation, were more likely to have attained complete remission at transplantation, and were more likely to receive tacrolimus for GVHD prophylaxis; unrelated cord blood recipients were older, underwent transplantation more recently, and were more likely to receive purine analog–containing conditioning regimens. All unrelated cord blood recipients received a single cord blood unit that was not manipulated ex vivo. The median weight of unrelated cord blood recipients was 52.0 kg (range, 31.0-90.2 kg); the median dose of nucleated cells and $CD34^+$ progenitor cells in the grafts, measured before freezing, was 2.55×10^7 (range, $1.39\text{-}5.34 \times 10^7$) and 0.79×10^5 (range, $0.07\text{-}3.15 \times 10^5$) per kg of recipient body weight, respectively.

Engraftment and GVHD

Of 310 patients who survived 30 days after transplantation and were evaluable for engraftment, primary graft failure was reported in 2 (6%) of 35 recipients of HLA-mismatched related grafts and in 12 (17%) of 70 recipients of unrelated cord blood, whereas the remaining 296 patients had evidence of initial engraftment. Acute GVHD of grades II, III, or IV occurred in 158 (47%) of 333

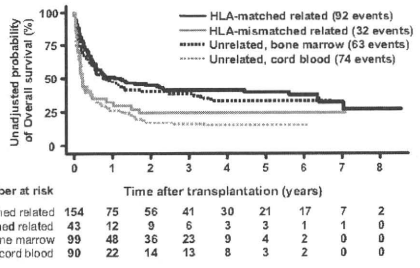

Figure 1. Unadjusted probability of overall survival according to type of graft source. The unadjusted Kaplan-Meier estimates of overall survival stratified according to type of graft source are shown.

evaluable patients; 69 (49%) of 140 HLA-matched related bone marrow or peripheral blood recipients, 20 (56%) of 36 HLA-mismatched related bone marrow or peripheral blood recipients, 40 (44%) of 91 unrelated bone marrow recipients, and 29 (44%) of 66 unrelated cord blood recipients. In a multivariable analysis, rates of grades II to IV acute GVHD did not significantly differ among the 4 groups (supplemental Table 1; available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Chronic GVHD occurred in 94 (48%) of 195 evaluable patients at a significantly lower rate among the unrelated cord blood recipients than among HLA-matched graft recipients (hazard ratio, 0.25; 95% CI, 0.10-0.61, $P = .002$).

Relapse and disease progression

Of 333 patients who survived 30 days after transplantation, 136 patients experienced relapse or progression of ATL at a median of 76 days (range, 1-1964 days) after transplantation. ATL recurred or progressed in 52 (37%) of 141 recipients of HLA-matched related grafts, in 19 (51%) of 37 recipients of HLA-mismatched related grafts, in 27 (32%) of 85 recipients of unrelated bone marrow, and 38 (54%) of 70 recipients of unrelated cord blood. Of 113 patients who were evaluable for the date of relapse or disease progression, the median time from transplantation to relapse or progression of ATL was 65.5 days (range, 1-1964 days) for HLA-matched related bone marrow or peripheral blood recipients, 63 days (range, 22-269 days) for HLA-mismatched related bone marrow or peripheral blood recipients, 152 days (range, 42-819 days) for unrelated bone marrow recipients, and 83 days (range, 7-596 days) for unrelated cord blood recipients.

Overall survival

Of 386 patients included in the study, a total of 125 patients were alive and 101 patients were alive in continuous complete remission after a median follow-up of 41 months (range, 1.5-102 months). The unadjusted 3-year probability of overall survival was 33% (95% CI, 28%-38%) for the whole cohort; 41% (95% CI, 33%-49%) in HLA-matched related graft recipients; 24% (95% CI, 12%-38%) in HLA-mismatched related graft recipients; 39% (95% CI, 29%-49%) in unrelated bone marrow recipients; and 17% (95% CI, 9%-25%) in unrelated cord blood recipients (Figure 1). The median overall survival time after transplantation was 9.8 months for HLA-matched related bone marrow or peripheral blood recipients, 2.5 months for HLA-mismatched related bone marrow or peripheral blood recipients, 9.6 months for unrelated bone marrow recipients, and 2.6 months for unrelated cord blood recipients. Patients who received transplants in complete remission had a higher probability of survival than those who received transplants not in complete remission (51% [95% CI, 41%-60%] vs 26% [95% CI, 20%-31%], $P < .001$). Multivariable analyses revealed 4 factors that adversely affected overall survival: older recipient age (> 50 years; hazard ratio, 1.56; 95% CI, 1.14-2.12, $P = .005$), male recipient (hazard ratio, 1.37; 95% CI, 1.07-1.77, $P = .014$), lack of complete remission at transplantation (hazard ratio, 2.01; 95% CI, 1.50-2.71, $P < .001$), and transplantation of unrelated cord blood. Hazard ratios for death among recipients of HLA-mismatched related transplants, unrelated bone marrow transplants, and unrelated cord blood transplants, compared with that among recipients of HLA-matched related transplants, were 1.55 (95% CI, 0.98-2.45, $P = .063$), 1.24 (95% CI, 0.82-1.88, $P = .312$), and 2.08 (95% CI, 1.43-3.02, $P < .001$), respectively (Table 2).

Treatment-related mortality and disease-associated mortality

Overall, 161 (43%) of 376 evaluable patients succumbed to treatment-related complications. Cumulative incidence of treatment-related mortality at 3 years after transplantation was 37% (95% CI, 29%-45%) in HLA-matched related bone marrow or peripheral blood recipients, 43% (95% CI, 28%-57%) in HLA-mismatched related bone marrow or peripheral blood recipients, 42% (95% CI, 32%-51%) in unrelated bone marrow recipients, and 52% (95% CI, 41%-62%) in unrelated cord blood recipients (Figure 2A). When adjusted by multivariable analysis, patients given unrelated cord blood (hazard ratio, 1.77; 95% CI, 1.10-2.86, $P = .019$) had higher treatment-related mortality rates (Table 2).

Deaths from progression of ATL occurred in 90 (24%) patients. Cumulative incidence of disease-associated mortality at 3 years after transplantation was 21% (95% CI, 14%-28%) in HLA-matched related bone marrow or peripheral blood recipients, 32% (95% CI, 19%-47%) in HLA-mismatched related bone marrow or peripheral blood recipients, 19% (95% CI, 12%-28%) in unrelated bone marrow recipients, and 30% (95% CI, 21%-40%) in unrelated cord blood recipients (Figure 2B). In multivariable analysis, patients given transplants not in remission (hazard ratio, 2.55; 95% CI 1.50-4.33, $P = .001$) or male recipients (hazard ratio, 1.86; 95% CI, 1.17-2.95, $P = .008$) had higher rates of disease-associated mortality (Table 2).

Causes of death after transplantation are summarized in Table 3. Of the 161 patients who died of treatment-related complications, 51 (32%) succumbed to infection and 53 (33%) to organ failure. Treatment-related events were principal causes of early death, whereas death from relapse or progression of ATL was more common later than 100 days after transplantation, irrespective of types of graft source.

Effect of donor HTLV-I serostatus on outcomes

Data on donor HTLV-I serostatus were available for analysis in 156 of 197 patients given related transplants; 68 received transplants from an HTLV-I–seropositive donor and 88 from an HTLV-I–seronegative donor. Patients who received transplants from HTLV-I–seropositive donors and those from HTLV-I–seronegative donors had similar background characteristics (supplemental Table 2). Among 113 patients who had data on donor HTLV-I serostatus and maintained or attained complete remission after transplantation, relapse of ATL was observed in 18 (38%) of 48 patients who received transplants from an HTLV-I–seropositive donor, and 16 (25%) of 65 patients who received transplants from an HTLV-I–seronegative donor with a median follow-up time for survivors of 40 months (range, 7.3-102 months). In univariable and

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ORIGINAL ARTICLE

Peripheral blood stem cell versus bone marrow transplantation from HLA-identical sibling donors in patients with leukemia: a propensity score-based comparison from the Japan Society for Hematopoietic Stem Cell Transplantation registry

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Abstract We retrospectively analyzed the results of 707 adult patients who underwent myeloablative peripheral blood stem cell transplantation (PBSCT) ($n = 365$) and myeloablative bone marrow transplantation (BMT) ($n = 342$) for leukemia from HLA-identical sibling donors between 2000 and 2005 using the propensity score method. The results were obtained from the Japan Society for Hematopoietic Cell Transplantation registry. Multivariate Cox analysis showed that PBSCT was associated with lower overall survival (OS) in standard-risk patients [adjusted hazard ratio (aHR) = 1.83; 95% confidence interval (CI) 1.04–3.23; $P = 0.036$], but not in high-risk patients (aHR = 1.11; 95% CI 0.76–1.61; $P = 0.599$). Hematopoietic recovery was significantly faster after

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PBSCT. The risk of acquiring grade III–IV acute graft-versus-host disease (GVHD) (aHR = 2.23; $P = 0.040$) and extensive chronic GVHD (aHR = 1.93; $P = 0.001$) were significantly higher after PBSCT. PBSCT was associated with higher non-relapse mortality in standard-risk patients (aHR = 2.30; 95% CI 1.08–4.88; $P = 0.030$), but not in high-risk patients (aHR = 1.29; 95% CI 0.65–2.54; $P = 0.468$). Relapse after transplantation did not differ between PBSCT and BMT either in standard-risk group or in high-risk group (aHR = 1.17; 95% CI 0.55–2.52; $P = 0.684$ and aHR = 0.81; 95% CI 0.52–1.28; $P = 0.370$, respectively). In this retrospective analysis, OS was significantly lower after PBSCT in standard-risk patients, but not in high-risk patients. PBSCT was associated with significant risks of grade III–IV acute GVHD and extensive chronic GVHD.

Keywords Bone marrow transplantation · Peripheral blood stem cell transplantation · Allogeneic · Graft-versus-host disease

1 Introduction

During the past decade, allogeneic peripheral blood stem cell transplantation (allo-PBSCT) has been increasingly used as an alternative to allogeneic bone marrow transplantation (allo-BMT) [1]. Furthermore, allo-PBSCT is associated with rapid hematopoietic recovery. Several prospective randomized controlled trials conducted in Western countries have shown an increased incidence of chronic graft-versus-host disease (GVHD) [2–11]. Nevertheless, there is still substantial controversy regarding survival, acute GVHD, non-relapse mortality (NRM), and relapse [12–14].

Ethnicity has been reported to affect the incidence and severity of GVHD [15]. Japanese patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) were found to have a lower incidence of acute GVHD than those from Western patients [16, 17]. Therefore, the outcome of allo-PBSCT compared with that of allo-BMT may differ according to the ethnic background.

Using the propensity score method, we retrospectively analyzed the clinical outcomes of 707 adult Japanese leukemia patients who received allogeneic HSCT with myeloablative conditioning from HLA-identical sibling donors. These data were obtained from the Japan Society for Hematopoietic Cell Transplantation (JSHCT) registry. A propensity scoring system was devised to estimate the effects of treatments by comparing outcomes of those subjects who were not randomly assigned to experimental or control groups in an observational study [18]. A randomized control trial is superior in eliminating the confounding factors of known and unknown covariates by random treatment assignment. The propensity score expresses the likelihood of being assigned to experimental or control treatments, and is calculated using logistic regression models, including variables measured prior to treatment as much as possible. Considering the propensity score in this analysis, we expected that a hypothetical evaluation of an experimental trial in an observational study would give results similar to those of an evaluation in a randomized controlled trial.

2 Patients and methods

2.1 Study population

Using a standardized reporting form, JSHCT collects data on individual transplant patients from each transplant center, and follow-up reports are submitted annually after transplantation. A total of 1,426 patients, who underwent allogeneic HSCT between 2000 and 2005 for acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), and chronic myelogenous leukemia (CML), have been reported to JSHCT. Patients were excluded from the study if their data were incomplete ($n = 205$), if they received a non-myeloablative or reduced-intensity conditioning regimen ($n = 223$), if they received grafts from other than HLA-identical siblings ($n = 217$), if they were less than 18 years of age ($n = 38$), if they had a previous history of HSCT ($n = 10$), and if they had non-allo-PBSCT or non-allo-BMT ($n = 16$). In Japan, most

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allo-HSCT patients have received granulocyte-colony stimulating factor (G-CSF) post-transplant [19]. The August 2006 data of the remaining 707 patients were analyzed. This study was approved by the Data Management Committee for the Nationwide Survey of JSHCT.

2.2 Definitions

Risk status at transplantation was categorized as either standard or high. Standard-risk diseases included acute leukemia in first complete remission (CR) and CML in first chronic phase (CP). Other disease status was categorized as high-risk disease [11]. The day of neutrophil engraftment was defined as the first of three consecutive days with an absolute neutrophil count (ANC) of more than 0.5×10^9/L. The day of platelet engraftment was defined as the first of seven consecutive days with a platelet count of more than 20×10^9/L without platelet transfusion. Acute GVHD was graded according to the standard criteria [20]. All patients who had no evidence of graft failure and survived beyond day 28 were considered to be evaluable for acute GVHD. GVHD persisting beyond day 100 and de novo GVHD occurring after day 100 were classified as chronic GVHD. The incidence of chronic GVHD was calculated in patients followed for more than 100 days, and the disease was classified as none, limited, or extensive [21]. Overall survival (OS) was defined as the duration of survival between transplantation and either death or the last follow-up.

Relapse was defined as disease progression with censored NRM. NRM included all causes of death other than relapse occurring at any time after transplantation. All deaths were considered in the estimation of OS.

2.2.1 Endpoints

The primary endpoint of comparison was OS. Secondary endpoints were hematopoietic recovery, acute GVHD (grade II–IV and III–IV), chronic GVHD (overall and extensive), NRM, and relapse.

2.2.2 Propensity score calculation

We calculated the propensity score using the pscore command in STATA version 10.1. (STATA, College Station, TX, USA) [22]. Factors included in the propensity score were as follows: age at HSCT in categories (<40, 40–49, and 50+) as an ordinal variable; sex (male/female) as an indicator variable; year of transplantation as a continuous variable; performance status at transplantation as an ordinal variable; risk status (CR1/CP1, CR2/CP2, or more advanced) as an indicator variable; a cumulative number of HSCT from related donors at an institution between 2000 and 2005 (1: 1–4, 62 institutions; 2: 5–11, 58 institutions; 3: 12 or more, 52 institutions) as an ordinal variable; and the percentage of allo-PBSCT out of total HSCT from HLA-identical siblings in tertile (1: <56%, 59 institutions; 2: 56–90%, 56 institutions; 3: 91% or more, 57 institutions) as an ordinal variable. We utilized as many variables as possible in the propensity score to evaluate the effects of known and unknown factors on the choice of treatment. After calculating the propensity score, the subjects were divided into four groups according to quartile. The numbers of subjects in quartiles 1–4 (allo-PBSCT/allo-BMT) were 23/154, 58/120, 126/50, and 158/18, respectively.

2.2.3 Statistical analysis

Patient characteristics and therapeutic outcomes were compared between allo-PBSCT and allo-BMT groups. OS was assessed using the Kaplan–Meier product limit method [23, 24]. Cumulative incidences of acute GVHD, chronic GVHD, NRM, and relapse were evaluated as 1 − (Kaplan–Meier estimate) instead of applying methods considering competing risks [25, 26] to maintain statistical consistency between logrank tests and methods of cumulative incidence estimation. Allo-PBSCT and allo-BMT groups were compared using the propensity score in quartiles [1–4], a stratified logrank test, and a stratified Cox proportional hazards model. Diagnosis (AML, ALL, and CML) and quartile of the propensity score were stratification factors. Confounders considered in the Cox proportional hazards model were as follows: year of diagnosis as a continuous variable; year of transplantation as a continuous variable; age at transplantation as a continuous variable; sex (male/female); sex matching (match/male to female/female to male/unknown); performance status (0, 1, 2, 3–4, and unknown); risk status (standard/high); GVHD prophylaxis [cyclosporin (CsA) + methotrexate (MTX), tacrolimus (TAC) + MTX, and others]; and conditioning regimen [total body irradiation (TBI)-containing regimen, busulfan and cyclophosphamide (BU/CY), and others]. All analyses were performed using STATA version 10.1, and P values less than 0.05 were considered statistically significant.

3 Results

3.1 Patient characteristics

The characteristics of patients are summarized in Table 1. The number of patients who underwent allo-PBSCT was 365, and that who underwent allo-BMT was 342. The median age at HSCT was 39 years (range 18–64 years) in the allo-PBSCT group and 39 years (range 18–59 years) in the allo-BMT group. The allo-PBSCT group included significantly more male patients from female donors than

Table 1 Characteristics of patients

	PBSCT *n* (%)	BMT *n* (%)	*P* value (Mann–Whitney test)
No. of patients	365	342	
Median patients age, years (range)	39, 18–64	39, 18–59	0.962
Patients sex (male/female)	210/155	189/153	0.543
Sex matching			
Matched	176 (48.2)	185 (54.1)	
Male to female	70 (19.2)	78 (22.8)	
Female to male	106 (29.0)	71 (20.8)	
Unknown	13 (3.6)	8 (2.3)	0.043
Risk group			
Standard-risk	149 (40.8)	202 (59.1)	
High-risk	216 (59.2)	140 (40.9)	<0.001
Diagnosis			
Standard-risk			
AML	58 (38.9)	76 (37.6)	
ALL	46 (30.9)	51 (25.2)	
CML	45 (30.2)	75 (37.2)	0.322
High-risk			
AML	128 (59.3)	75 (53.6)	
ALL	58 (26.9)	28 (20.0)	
CML	30 (13.8)	37 (26.4)	0.026
Performance status			
0	185 (50.7)	138 (40.4)	
1	73 (20.0)	55 (16.1)	
2	24 (6.6)	16 (4.7)	
3 or 4	12 (3.3)	2 (0.6)	
Unknown	71 (19.5)	131 (38.3)	<0.001
Conditioning regimen			
TBI-based	225 (61.6)	205 (59.9)	
Bu/CY	110 (30.1)	118 (34.5)	
Others	30 (8.3)	19 (5.6)	0.23
GVHD prophylaxis			
CsA + MTX	308 (84.4)	300 (87.7)	
TAC + MTX	12 (3.3)	14 (4.1)	
Others	45 (12.3)	28 (8.2)	0.176

Standard-risk diseases: acute leukemia in first complete remission and chronic myelogenous leukemia in first chronic phase; other disease status was categorized as high-risk diseases

PBSCT peripheral blood stem cell transplantation, *BMT* bone marrow transplantation, *AML* acute myelogenous leukemia, *ALL* acute lymphoblastic leukemia, *CML* chronic myelogenous leukemia, *TBI* total body irradiation, *Bu* busulfan, *CY* cyclophosphamide, *GVHD* graft-versus-host disease, *CsA* cyclosporin, *MTX* methotrexate, *TAC* tacrolimus

the allo-BMT group (Mann–Whitney test, $P = 0.043$). AML, ALL, and CML were diagnosed in 337, 183, and 187 patients, respectively. The allo-PBSCT group included significantly more high-risk patients than the allo-BMT group ($P < 0.001$). Among the high-risk patients, the allo-BMT group had significantly more CML patients than the allo-PBSCT group ($P = 0.026$). Conditioning regimen and GVHD prophylaxis were performed according to the protocol of each institution, and there were no differences between the two groups. The most frequently used conditioning regimens were BU/CY (busulfan 1 mg/kg × 4/day × 4 days with cyclophosphamide 60 mg/kg/day × 2 days) and CY/TBI (cyclophosphamide 60 mg/kg/day × 2 days with total body irradiation 10–12 Gy). CsA plus MTX was used most frequently for GVHD prophylaxis. Median follow-up period for the surviving patients at the time of analysis was 33 months (1.8–55 months) in the PBSCT group and 31 months (1–53 months) in the BMT group.

3.2 Primary endpoint

3.2.1 Overall survival

Three-year OS in standard-risk patients was 68% [95% confidence interval (CI) 59–75] after allo-PBSCT and 77%

(95% CI 70–82) after allo-BMT (by disease and quartile in the propensity-score stratified logrank test; $P = 0.023$). Three-year OS in high-risk patients after allo-PBSCT and allo-BMT was 38% (95% CI 31–45) and 54% (95% CI 44–62), respectively ($P = 0.587$) (Fig. 1). Multivariate Cox analysis showed that allo-PBSCT was a significant factor for lower OS in the population with standard-risk [adjusted hazard ratio (aHR) = 1.83; 95% CI 1.04–3.23; $P = 0.036$], but not that with high-risk (aHR = 1.11; 95% CI 0.76–1.61; $P = 0.599$).

3.3 Secondary endpoints

3.3.1 Hematopoietic recovery

Engraftment occurred in all patients receiving allo-PBSCT and allo-BMT (allo-PBSCT, $n = 324$; allo-BMT, $n = 305$) surviving for more than 28 days. Allo-PBSCT patients showed significantly faster neutrophil and platelet recovery compared with allo-BMT patients. The median time of recovery to ANC $> 0.5 \times 10^9$/L was 14 days for the allo-PBSCT group and 16 days for the allo-BMT group, respectively (stratified logrank test, $P < 0.0001$). The median time of recovery to a platelet count $>20 \times 10^9$/L was 15 days for the allo-PBSCT group and 21 days for the allo-BMT group, respectively ($P < 0.0001$). In the multivariate Cox analysis, allo-PBSCT was a significant factor for faster neutrophil (aHR = 0.57; 95% CI 0.45–0.71; $P < 0.001$) and platelet (aHR = 0.56; 95% CI 0.44–0.71; $P < 0.001$) recovery compared with allo-BMT.

3.3.2 Acute GVHD

The cumulative incidence of grade II–IV acute GVHD was 31% (95% CI 27–35) in all patients, whereas that in allo-PBSCT and allo-BMT groups was 35% (95% CI 30–41) and 26% (95% CI 22–32) (stratified logrank test, $P = 0.221$), respectively. The aHR for grade II–IV acute GVHD after allo-PBSCT was 1.25 (95% CI 0.85–1.84; $P = 0.260$) by multivariate Cox analysis. The cumulative incidence of grade III–IV acute GVHD was 14% (95% CI 10–18) and 5.4% (95% CI 3.3–8.8) in the allo-PBSCT and allo-BMT groups, respectively ($P = 0.021$). Multivariate Cox analysis showed that allo-PBSCT was a significant factor for the development of grade III–IV acute GVHD (aHR = 2.23; 95% CI 1.04–4.78; $P = 0.040$; Fig. 2).

3.3.3 Chronic GVHD

The risk of chronic GVHD in the first year after transplantation was significantly higher after allo-PBSCT than after allo-BMT (cumulative incidence at 1 year, 51%; 95% CI 44–58 after allo-PBSCT vs. 34%; 95% CI 28–41 after allo-BMT; $P = 0.0005$ with stratified logrank test). The extensive form of chronic GVHD was more prevalent in the allo-PBSCT group than in the allo-BMT group (26%; 95% CI 21–33 with allo-PBSCT and 15%; 95% CI 11–20 with allo-BMT; $P = 0.0017$). Multivariate Cox analysis showed that allo-PBSCT was a significant factor for the development of extensive chronic GVHD (aHR = 1.93; 95% CI 1.32–2.84; $P = 0.001$; Fig. 3).

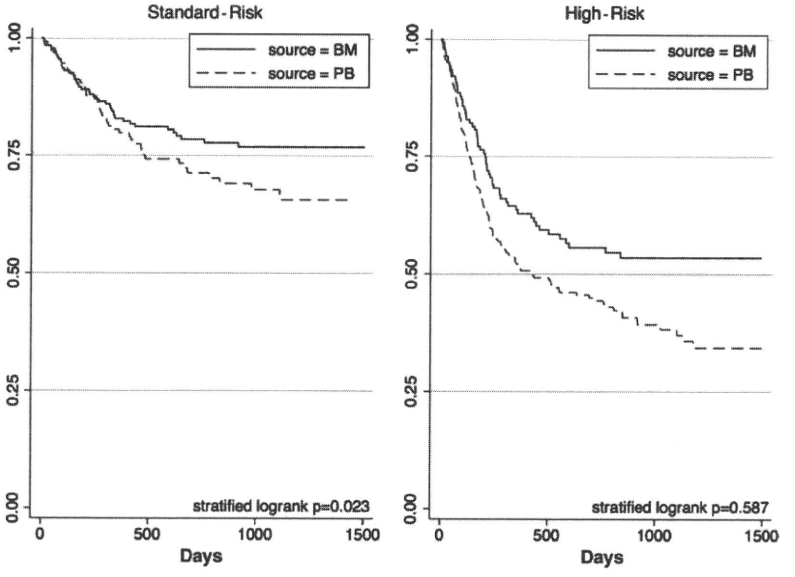

Fig. 1 Probabilities of overall survival after peripheral blood stem cell transplantation compared with bone marrow transplantation. Standard-risk diseases included acute leukemia in first complete remission and chronic myelogenous leukemia in first chronic phase. Other diseases were categorized as high-risk diseases

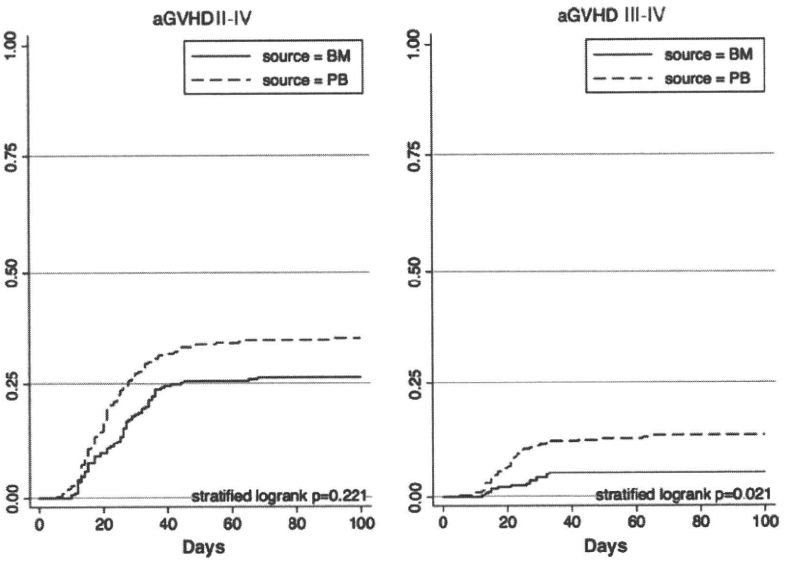

Fig. 2 Probabilities for grade II–IV and III–IV acute graft-versus-host disease (GVHD) after peripheral blood stem cell transplantation compared with bone marrow transplantation

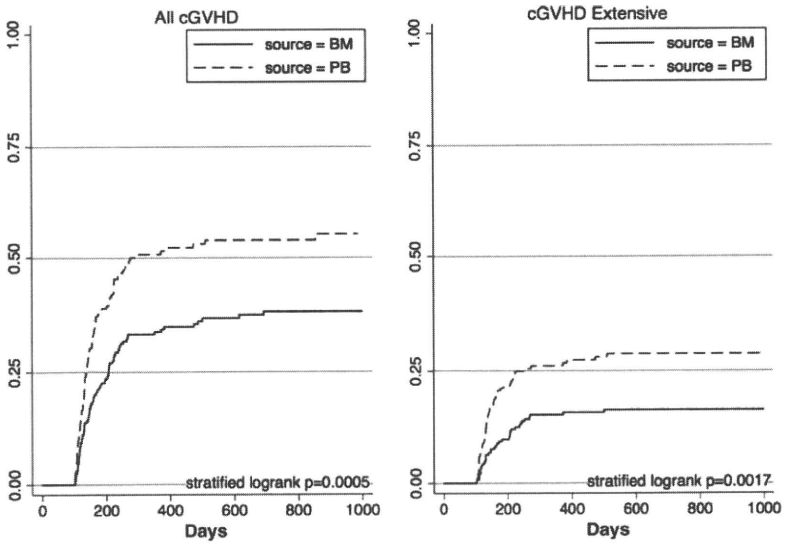

Fig. 3 Probabilities for chronic GVHD and extensive chronic GVHD after peripheral blood stem cell transplantation compared with bone marrow transplantation

3.3.4 Non-relapse mortality

The cumulative incidence of NRM for the standard-risk group at day 100 was 4.7% (95% CI 2.3–9.7) after allo-PBSCT and 6.0% (95% CI 3.4–10.2) after allo-BMT, and that at 1 year was 14.2% (95% CI 9.4–21.1) after allo-PBSCT and 11.2% (95% CI 8.0–17.2) after allo-BMT (stratified logrank test, $P = 0.047$). The cumulative incidence of NRM for the high-risk group at day 100 was 11.2% (95% CI 7.6–16.4) after allo-PBSCT and 8.9% (95% CI 5.1–15.1) after allo-BMT, and that at 1 year was 24.4% (95% CI 18.7–31.4) after allo-PBSCT and 14.7% (95% CI 9.6–22.2) after allo-BMT (stratified logrank test, $P = 0.221$) (Fig. 4).