reconstituted in xenogenic hosts for a long-term in our and others' experiments (42), it is highly likely that $hCD34^+hCD38^-hLin^-$ population is highly enriched for hLT-HSCs. Therefore, it is suggested that the negative expression of hFlt3 does not mark LT-HSCs in human, while mFlt3 does in mouse (16, 17). Second, in contrast to mouse hematopoiesis, where mFlt3 expression is restricted within progenitor populations of lymphoid potential including CLPs and a minority of CMPs that can differentiate into B cells (20), hFlt3 is expressed in human CMPs and GMPs, as well as in CLPs. The Flt3 expression is suppressed after cells are committed into the MegE lineage in both human and mouse. The distribution of Flt3 in mouse and human hematopoiesis is schematized in Fig. 7. The significant difference of Flt3 distribution in human and mouse hematopoiesis suggests that the critical role of Flt3 signaling in hematopoietic development could also be different between these species.

We further found that the important function of hFlt3 should include the maintenance of cell survival via the up-regulation of anti-apoptotic Mcl-1 in early hematopoiesis. Previous studies have demonstrated that FL can support in vitro survival of human long-term culture-initiating cells (24, 46, 47). MCL-1 is a non-redundant anti-apoptotic protein, at least in mouse hematopoiesis, because the removal of Mcl-1 from hematopoietic cells in a conditional knockout system caused fatal hematopoietic failure, and because in vitro disruption of *Mcl-1* in mouse HSCs, CMPs, or CLPs rapidly induced their apoptotic cell death (45). The expression level of Mcl-1 was the highest at the HSC stage and gradually declined as HSCs differentiate into myeloid and lymphoid progenitors in mouse hematopoiesis (45). The pattern of Mcl-1 distribution is well preserved in human hematopoiesis (Fig. 6A), suggesting that Mcl-1 might also be essential for hHSC survival. In mouse HSCs, Mcl-1 is up-regulated by signals from cytokines including SCF, IL-6, and IL-11, and SCF exerts the most potent effect on the up-regulation of Mcl-1(45). In contrast to mouse LT-HSCs that express c-Kit but not Flt3, functional hLT-HSCs coexpress c-Kit and Flt3 (Fig. 1), and importantly, FL as well as SCF are potent inducers for Mcl-1 transcription (Fig. 6). The fact that FL and SCF activated only Mcl-1, but not Bcl-2 or Bcl-x_L, in turn suggests that Mcl-1 might be the most critical survival factor controlled by exogenous cytokine signals at the HSC stage. Although it remains unclear whether hFlt3 and/or c-Kit signaling is absolutely required for hHSC survival, our data suggest that, to maintain the Mcl-1 level in hHSCs, the Flt3/FL system could work as an alternative to the SCF/c-Kit system. This is of interest because the SCF/c-Kit system is non-redundant in mouse hematopoiesis (48), where mouse LT-HSCs express only c-Kit, but not Flt3.

The anti-apoptotic effect of hFlt3 signaling was also seen in hFlt3-expressing myeloid progenitor populations. The incubation of CMPs and GMPs with FL significantly prevented their apoptotic cell death in vitro, and FL, as well as SCF, rapidly activated the Mcl-1 transcription in these progenitors. Interestingly, in CLPs, FL activated not only Mcl-1 but also Bcl-2. In lymphopoiesis, Bcl-2 (49, 50), as well as Mcl-1 (51), is critical. FL may collaborate with IL-7 to maintain lymphoid cell survival by up-regulating both Bcl-2 and Mcl-1. Collectively, in humans, Flt3 signaling might support cell survival in early hematopoietic stages with only the exception of the MegE lineage developmental pathway.

Our data also provides an important insight into pathogenesis of AML with *FLT3* mutations. A total of 15–35% of AML patients have either internal tandem duplications (ITDs) in the juxtamembrane domain or mutations in the activating loop of *FLT3* (28, 29), resulting in ligand-independent constitutive signal activation. The *FLT3* mutations are rarely found in acute lymphoblastic leukemia (28, 29). The etiologic link of *FLT3* mutations with AML does not fit the lymphoid-only expression pattern of Flt3 in mouse hematopoiesis. In mouse models, however, the ectopic expression of *FLT3*-ITDs in the bone marrow promotes development of myeloproliferative disorders, but these mutations themselves do not cause leukemia (52). We have found that AML cells with *FLT3*-ITD mutations possess extremely high levels of Mcl-1, and transduction of *FLT3*-ITD into normal HSCs induces rapid up-regulation of Mcl-1 of up to >10-fold higher levels (G. Yoshimoto and K. Akashi, manuscript in preparation). Because the expression of *FLT3* mutations should occur in concert with that of normal Flt3, our data suggest that once *FLT3* mutations are acquired in human hematopoiesis, abnormal survival-promoting signals of Mcl-1 should be expressed in LT-HSCs, and is progressively up-regulated in GMPs. It has been shown that both LT-HSCs and GMPs are the critical cellular target for leukemic transformation. The reinforced survival of CMPs/GMPs by blocking two independent apoptotic pathways (53), or the enforced expression of bcr-abl together with survival-promoting Bcl-2 at the GMP stage (54), results in AML development in mouse models. In human bcr-abl-positive chronic myelogeneous leukemia, GMPs could be the target for blastic transformation by acquisition of β-catenin signaling (55). GMPs can also be converted into leukemic stem cells simply by transducing leukemia fusion genes, such as MLL-ENL (56) or MOZ-TIF2 (57). Thus, these data collectively suggest that the acquisition of *FLT3* mutations in human hematopoiesis might induce the reinforced survival of cells at the HSC and myeloid progenitor stages, where *FLT3* mutations might collaborate with other genetic abnormalities to achieve full AML transformation.

In conclusion, our data show that the distribution of Flt3 is quite different in mouse and human hematopoeisis. hFlt3 targets LT-HSCs and myeloid progenitors except for MEPs. Flt3 signaling might support cell survival in early hematopoiesis including the HSC and the myeloid progenitor stages through up-regulation of Mcl-1. This is a striking example that the expression pattern of key molecules could be significantly different between human and mouse. Accordingly, special considerations are required in using mouse models to understand the role of Flt3 and *FLT3* mutations in human hematopoiesis.

Disclosures

The authors have no financial conflict of interest.

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Case report

Disseminated tuberculosis following second unrelated cord blood transplantation for acute myelogenous leukemia

T. Shima, G. Yoshimoto, T. Miyamoto, S. Yoshida, K. Kamezaki, K. Takenaka, H. Iwasaki, N. Harada, K. Nagafuji, T. Teshima, N. Shimono, K. Akashi. Disseminated tuberculosis following second unrelated cord blood transplantation for acute myelogenous leukemia. Transpl Infect Dis 2009: **11**: 75–77. All rights reserved

Abstract: Here we report the case of a 43-year-old Japanese woman with acute myelogenous leukemia who underwent 2 unrelated cord blood transplantations (UCBT), terminating in fatal disseminated tuberculosis (TB). The patient did not achieve remission despite intensive chemotherapy, and subsequently underwent UCBT with a standard conditioning regimen. However, engraftment was not achieved. Fifty days after the first UCBT, the patient underwent a second UCBT with a reduced-intensity conditioning regimen. She developed a pre-engraftment immune reaction, which responded well to prednisolone, and engraftment was documented. However, 50 days after the second UCBT, the patient presented with high fever and developed pneumonia despite antibiotic and antifungal treatments. Thereafter, *Mycobacterium tuberculosis* was detected in blood cultures and specimens of bronchoalveolar lavage, thus indicating disseminated TB. Despite anti-tuberculous treatment, she died on day 85. TB should always be considered as a possible diagnosis when treating febrile immunocompromised patients.

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Key words: tuberculosis; disseminated; cord blood; transplantation; AML

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Unrelated cord blood transplantation (UCBT) has been established as an alternative to allogeneic hematopoietic stem cell transplantation (allo-HSCT). UCBT can provide potential advantages in the form of rapid availability and a lower risk of graft-versus-host disease (GVHD), thus permitting less stringent human leukocyte antigen (HLA) matching (1). Furthermore, reduced-intensity conditioning (RIC) regimens for allo-HSCT can be undertaken as alternatives to conventional myeloablative conditioning regimens to decrease regimen-related toxicity while preserving anti-tumor effects (2). Therefore, the use of UCBT with RIC has been increasing recently for patients who do not have an HLA-matched donor, but have a history of prior transplantation or comorbid organ conditions to preclude the use of conventional allo-HSCT (3). However, despite several advantages and considerable progress in supportive care, opportunistic infections still remain a major cause of morbidity and mortality due to delayed or impaired immune reconstitution after UCBT (3).

Mycobacterium tuberculosis is a common pathogen worldwide. It infects one-third of the world's population, and is especially endemic in East Asia including Japan (4, 5). Recent reports on tuberculosis (TB) following HSCT have shown that this is a significant problem in endemic countries (6, 7). Here, we describe a case of disseminated TB manifesting early after a second UCBT with RIC for refractory acute myelogenous leukemia (AML). Immunologic immaturity of the infused UCB cells could result in a lack of tuberculous granulation formations, leading to fatal disseminated TB early after UCBT. It should be noted that if patients receiving HSCT have unexplained fever, tests to detect *M. tuberculosis* should be performed immediately to improve the outcome in these patients.

Case report

In August 2007, a 43-year-old Japanese woman was referred to our hospital because of hematologic abnormalities. Bone marrow aspiration and cytogenetic analysis revealed AML of the M2-subtype according to the French–American–British classification, with *t*(6, 9) abnormality. She did not achieve hematologic remission despite the induction of chemotherapy with idarubicin and cytosine arabinoside. In October 2007, she underwent UCBT with HLA mismatch at 2 loci. Cord blood cells (2.15×10^7 nucleated cells/kg and 1.32×10^5 CD34-positive cells/kg) were infused after a conditioning regimen including total body irradiation (TBI) (12 Gy) and cyclophosphamide (120 mg/kg). Prophylaxis for GVHD consisted of cyclosporine and short-term methotrexate. Hematopoietic recovery was delayed, and engraftment failure was confirmed by chimerism analysis using DNA amplification of polymorphic short tandem repeats of bone marrow cells on day 26.

Following this, in December 2007, 50 days after the first UCBT, she underwent the second UCBT from another donor with HLA mismatch at 2 loci. Cord blood cells (2.43×10^7 nucleated cells/kg and 1.03×10^5 CD34-positive cells/kg) were infused following a RIC regimen including fludarabine (125 mg/kg) and melphalan (80 mg/kg), and GVHD prophylaxis with cyclosporine and mycophenolate mofetil. On day 26, after the second UCBT, she presented with high fever, skin eruptions, and weight gain. She was diagnosed with a pre-engraftment immune reaction (8), which responded well to treatment with prednisolone (1 mg/kg daily). The patient became afebrile and engraftment was documented on day 33. On day 50, she developed high fever again, and chest computed tomography (CT) scan demonstrated homogenous amorphous opacification with air bronchograms in the right upper lobe, and an absence of the typical cavitary consolidation of TB (Fig. 1). The lesions were considered to be bacterial or fungal infections based on consultation with the radiologists and infectious disease control team. Despite the administration of broad-spectrum antibiotics and antifungal drugs, the high fever persisted. On day 55, Ziehl–Neelsen staining and polymerase chain reaction analysis of bronchoalveolar lavage specimens exhibited positivity for *M. tuberculosis* and negativity for other bacteria, fungi, *Pneumocystis jirovecii*, and cytomegalovirus. The subsequent blood culture was positive for *M. tuberculosis*, indicating that she suffered from disseminated TB. She had no prior history of TB. Transbronchial lung biopsy could not be performed because of an extremely low platelet count. Anti-tuberculous therapy with isoniazid, rifampicin, ethambutol, and pyrazinamide was started immediately. However, her respiratory state gradually worsened. Progression of hypoxia could not be controlled and finally she died on day 85. Postmortem examination was not permitted.

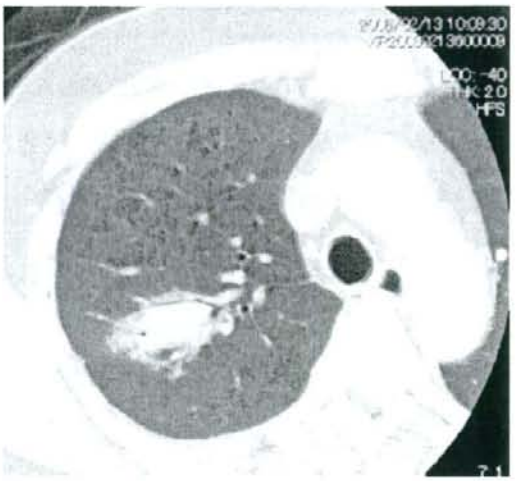

Fig. 1. Computed tomography image on day 50 showing consolidation in the right upper lobe, and homogenous amorphous opacification with air bronchograms, but not showing the typical cavitary consolidation of tuberculosis.

Discussion

The high incidence of TB in HSCT recipients is considered to be due to the severe immunodeficiencies that these patients suffer (6, 9). According to recent studies, TB occurred in about 0.4% of HSCT recipients; the incidence was reported as 0.13% in autologous and 0.57% in allo-HSCT, whereas mortality rates from TB amounted to approximately 0% in autologous and 30% in allogeneic patients, indicating that allo-HSCT presented a higher risk for the occurrence and greater severity of TB (10). Chronic GVHD, immunosuppressive therapy, TBI, and T-cell depletion are all risk factors contributing to the development of TB following allo-HSCT (6, 10, 11). Our patient underwent UCBT twice at a short interval. Conditioning regimens included TBI in the first UCBTand potent immunosuppressive agents such as fludarabine and melphalan were included in the RIC regimen for the second UCBT. In addition, the patient developed pre-engraftment immune reaction, which required prednisolone administration. Prolonged immunosuppression and damage to alveolar macrophages by TBI might have contributed to the onset of fatal TB (12).

Generally, TB lesions are more commonly restricted to lungs; however, in some cases, disseminated TB can occur (6, 10, 11). The patient in our report initially presented with disseminated TB, despite having no prior history of TB. Only one report documents cases of TB following UCBT (13). The group in Toranomon, Japan, reported that TB was diagnosed in 3 out of 113 (2.7%) adult patients, and all 3 manifested disseminated TB at diagnosis (13). Despite anti-tuberculous treatment, 2 patients died immediately. This report, together with our case, suggests that UCBT recipients might have a higher risk for disseminated TB and a higher mortality rate than transplantation recipients having another stem cell source. Under steady-state conditions, T cells and macrophages secrete interferon-gamma and tumor necrosis factor-alpha, which are crucial mediators of protection against the onset of *M. tuberculosis*-mediated granuloma (14–16). However, cord blood T cells and macrophages represent a naive and immunologic immature cell population to be primed for defense against infections including TB (17). The Toranomon group also reported that biopsy specimens collected from UCBT recipients with TB revealed necrosis without granulation in any organ (13). In our case, CT images showed consolidation reflecting bacterial or fungal infections, but did not show the typical cavitary consolidation of TB. Demirkazik et al. (18) reported that in the evaluation of febrile immunocompromised patients on the basis of CT findings, pulmonary fungal infection and *P. jirovecii* pneumonia could be identified with great accuracy, but not TB. Therefore, the lack of tuberculous granulation formation due to the immaturity of cord blood cells can make the diagnosis of TB difficult based on the CT findings, and can result in the development of fatal disseminated TB following UCBT.

We described a UCBT patient who developed disseminated TB. In endemic areas including Japan, TB should be considered in HSCT patients with fever of unknown origin, and precise tests for *M. tuberculosis* detection should be conducted immediately. In addition, even if patients have had no past history of TB, prophylactic anti-tuberculous therapy could be considered in the event of severe immunosuppressive conditions.

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