Non-myeloablative bone marrow transplantation (NM-BMT) is a newly developed therapeutic strategy for malignant and non-malignant hematological diseases, as well as immunotherapy-responsive solid tumors. The graft- versus-leukemia (GVL) or graft- versus-tumor (GVT) effect is the major therapeutic effect of this procedure, which also decreases transplant-related mortality (TRM) while remaining relatively safe for older patients. Graft rejection may be a main concern for NM-BMT in high-risk patients such as unrelated-donor BMT and elder recipients, however, very few literatures have mentioned this issue. Here we report 2 cases of NM-BMT where delayed rejection developed after initial engraftment. The first case was a victim of chronic myelogenous leukemia (CML) in chronic phase receiving HLA-matched unrelated-donor (MUD) BMT using the non-myeloablative regimen (fludarabine/busulphan/ATG). Chimerism study after BMT revealed successful initial engraftment, however, pancytopenia developed since day +38. Bone marrow examination on day +47 revealed only 15% of donor-type cells, with subsequent salvage haploidentical BMT failing to engraft. The patient expired on day +71. The second case was a victim of myelodysplastic syndrome, received HLA-matched sibling-donor allogeneic BMT using the same regimen as for Case 1, with successful initial engraftment proved by chimerism study. Pancytopenia was noted since day +124, and chimerism study on day +127 revealed only 25% of donor-type cells. The patient expired on day +151. We recommend that the suitability of NM-BMT for high-risk patients such as unrelated-donor BMT and elder recipients needs further studies to confirm.
cessful engraftment and further T-lymphocyte-mediated immunotherapy in NM-BMT. However, there may be a higher frequency of graft failure after NM-BMT in high-risk patients such as unrelated-donor BMT and elder recipients, but very few literatures have addressed this issue. Here we report 2 cases of NM-BMT where delayed rejection developed after the initial engraftment.

**CASE REPORTS**

**Case 1**

A 21-year-old female was diagnosed with chronic myelogenous leukemia (CML) in December 1999, with initial presentation of leukocytosis and splenomegaly. She was also a victim of Hodgkin’s disease (stage IIIB, mixed cellularity type) diagnosed at the age of 18 years. Chemotherapy with 6 courses of standard ABVD regimen (doxorubicin, bleomycin, vinblastine and dacarbazine) and radiotherapy were prescribed at that time, with complete remission achieved until the CML was diagnosed. Bone marrow examination revealed CML in chronic phase, and chromosome study proved positive for Philadelphia chromosome. Oral hydroxyurea was administered after diagnosis and HLA-matched unrelated-donor (MUD) BMT was arranged because no HLA-matched sibling-donor (MSD) was available, with MUD-BMT performed on May 15, 2000. Non-myoeloblative conditioning regimen was selected because of her previous chemoradiotherapy history and the possibility of intolerance to traditional myeloablative regimen, and the protocol was according to the Slavin’s report in 1998: 4 fludarabine (Fludara; Schering AG, Berlin, Germany) 30 mg/m² once daily i.v. for 6 consecutive days (days -10 to -5; total dose 180 mg/m²); busulphan 4 mg/kg p.o. in divided daily doses for 2 consecutive days (days -6 to -5; total dose 8 mg/kg); anti-T lymphocyte globulin (ATG-Fresenius, Munich, Germany) 10 mg/kg i.v. for 4 consecutive days (days -4 to -1; total dose 40 mg/kg); cyclosporine-A (CSA) 1.5 mg/kg i.v. twice daily from day -1 for graft-versus-host-disease (GVHD) prophylaxis. The bone marrow cell dose was as follows: total cell count 2.10 10⁸ cells/kg, CD34+ cells 1.97 10⁶ cells/kg, and colony-forming unit cells (CFU-C) count 6.54 10⁴ cells/kg. Successful myeloid engraftment (first of 3 consecutive days on which the absolute neutrophil count (ANC) exceeded 0.5 10⁹ cells/L) was noted from day +15, however, platelet recovery (first of 7 consecutive days with an untransfused platelet count greater than 50 10⁹/L) was not achieved. Bone marrow examination on day +23 revealed successful three-lineage engraftment. Chimerism study using short-tandem-repeat (STR) analysis of bone marrow mononuclear cells (MNC) revealed full donor chimerism on day +23, and negative bone marrow BCR-ABL gene rearrangement on day +23. No symptoms or signs of acute GVHD were noted during whole course of transplantation.

Unfortunately, pancytopenia was detected on day +38 during regular follow-up (WBC 0.07 10⁹/L; hemoglobin 7.7 g/dL; platelet 20 10⁹/L). Bone marrow examination on day +47 revealed severe aplasia with very few hematopoietic elements, and graft failure was considered. Subsequent chimerism study on the same day revealed only 15% donor-type cells, and a series of cytomegalovirus (CMV) studies, including serum IgM and urine CMV-PCR, to rule out the possibility of CMV-related rejection, proved negative. Due to persistent aplasia, salvage haploidentical BMT donated by the patient’s father was performed on day +61, however, it failed to engraft. The patient expired on day +71 due to sepsis with multi-organ failure. The hemogram data and dynamic chimerism study during whole course of transplantation was illustrated as Fig. 1.

**Case 2**

In July 1999, a 53-year-old woman was diagnosed with myelodysplastic syndrome-refractory anemia with excess of blasts in transformation (MDS-RAEBT). The initial presentation was leukocytosis and thrombocytopenia. Bone marrow examination revealed dysmyelopoiesis and dysmegakaryopoiesis, with a blast proportion of approximately 25%. Induction chemotherapy, consisting of seven consecutive days of cytarabine 200 mg/m² continuous i.v. infusion over 24 hours, together with daunomycin 45 mg/m² i.v. once daily for 3 consecutive days, was administered after diagnosis, with complete remission achieved. Consolidation chemotherapy, consisting of 2 courses of cytarabine plus
Fig. 1. The changes of white blood cell (WBC) and platelets count and the dynamic chimerism study during bone marrow transplantation of Case 1.

Fig. 2. The changes of white blood cell (WBC) and platelets count and the dynamic chimerism study during bone marrow transplantation of Case 2.
daunomycin, and 1 course of high-dose cytarabine plus mitoxantrone, was administered during August to November 1999. MSD-BMT (from older brother) was performed on December 29, 1999, using the same conditioning regimen detailed for Case 1 above. The bone marrow cell dose was as follows: total cell count 4.07 \(10^8\) cells/kg, CD34+ cells 4.77 \(10^6\) cells/kg, and CFU-C 9.85 \(10^4\) cells/kg. Successful myeloid engraftment was confirmed on day +14, and platelet recovery was reached on day +36. Bone marrow examination on day +14 revealed successful three-lineage engraftment, and chimerism study using STR analysis of bone marrow MNC confirmed full donor chimerism on the same day. No symptoms or signs of acute or chronic GVHD were noted during whole course of transplantation.

Unfortunately, progressive pancytopenia was found (WBC 0.6 \(10^9\)/L, hemoglobin 3.9 g/dL, platelet 27 \(10^9\)/L) on day +124 during regular follow-up. Bone marrow examination on day +127 revealed severe aplastic marrow with very few hematopoietic elements, and graft failure was considered. Chimerism study on day +127 revealed only 25% donor-type cells. In order to rule out the possibility of CMV-infection-related graft rejection, a series of CMV studies including serum IgM and urine CMV-PCR were performed, with negative results determined for all. ATG and high-dose methylprednisolone therapy were administered for 5 consecutive days from day +142 without effect. Finally, the patient expired on day +151 due to sepsis with multi-organ failure. The hemogram data and chimerism study during transplantation was shown in Fig. 2.

**DISCUSSION**

Graft rejection is the main concern for allogeneic BMT, and extensive studies have investigated the associated risk factors. In general, primary rejection should be considered rejection of marrow allograft by residual immunocompetent cells, occurring after pretransplantation cytoreductive therapy. Delayed rejection could be regarded as failure of functional hematopoiesis after occurrence of transient hematopoiesis, possibly due to myelosuppression from medication or infection, but more often mediated by immunological factors. The major risk factors for graft failure are HLA disparity, source of infused stem cells, type of immunosuppressants, medication that might interfere normal hematopoiesis, numerous blood-product transfusions before BMT, and post-BMT infection such as CMV infection. Further, a higher rejection rate has also been reported for MUD-BMT compared to MSD-BMT. Davies et al. analysed the clinical outcome for 108 patients that had undergone allogeneic BMT from siblings or unrelated donors. Significantly elevated primary rejection rate was determined for partially serologically matched unrelated-donor BMT compared to MSD-BMT or fully HLA-matched unrelated-donor procedures, however, delayed rejection was significantly more frequent for unrelated-donor BMT, regardless of whether serological HLA was partially or fully matched. A reasonable explanation involves the importance of major HLA loci in early engraftment, where the minor HLA antigens may indeed be targets of graft rejection, limiting sustained engraftment. Davies et al. have reported that HLA compatibility, younger age, male donor, negative CMV serology, higher cell dose, and absence of methotrexate in GVHD prophylaxis were associated with more rapid myeloid engraftment in unrelated-donor BMT.

The possible causes for delayed rejection in our cases are undetermined, however, several possibilities should be considered. In Case 1, the non-myeloablative transplantation may have influenced the result of the engraftment in MUD-BMT. Considering the impact of conditioning regimen of NM-BMT on engraftment, most regimens containing purine analogues or ATG could achieve a more than 90% engraftment rate. Unrelated donor seems to influence the engraftment results greater than the conditioning regimen used (Table 1). Bornhäuser et al. reported an unusual high frequency of delayed rejection (8/42) in patients receiving MUD NM-BMT with the preconditioning regimen of fludarabine/busulfan/ATG, and Maris et al. also reported a preliminary data of fludarabine-TBI based unrelated-donor NM-BMT with a 11.1% delayed rejection rate. However, the report regarded the probability that non-myeloablative conditioning may be associated with higher incidence of graft failure in MUD-BMT is still very limited. From the unpublished, limited experi-
ence gathered at our institution, and unusual high incidence of graft failure has been noted using fludarabine/busulfan/ATG as conditioning regimen for NM-BMT, i.e., 2 of 8 patients developed delayed rejection, and this phenomenon is compatible with the reports from Bornhäuser and Maris.22,23 In Case 2, the relatively advanced age may have contributed to the failure of normal hematopoietic function after successful initial engraftment, as older age has been associated with higher incidence of graft rejection after BMT.12 Although NM-BMT is usually recommended for older patients because of its relative safety due to reduced conditioning-regimen dose intensity and decreased TRM,9-11 the age factor may influence the engraftment outcome after NM-BMT.

Cell dose may also influence the engraftment kinetic greatly: Mavroudis et al. reported that for allogeneic transplants, patients received less than 1 × 10^6 cells/kg CD34+ cells were associated with a significant higher TRM rate, and more than 2 × 10^6 cells/kg CD34+ cells showed significantly earlier recovery of hematopoietic cells.29 According to this report, the CD34+ cell doses were sufficient for our 2 cases, so inadequate cell dose induced graft failure was unlikely. Donor leukocytes infusion (DLI) was not used in our cases because the full donor chimerism was reached shortly after NM-BMT. When chimerism was lost, DLI was also not used due to unavailability of donors.

Although the cause of delayed rejection in our cases is undetermined, it seems reasonable to question the suitability of non-myeloablative regimen for patients who are at a higher risk of graft failure, such as recipients of MUD and aged patients.

**ACKNOWLEDGMENTS**

This work was supported by the grants from Research Project No. 90-325 of Taipei Veterans General Hospital.

**REFERENCES**

5. Grigg A, Barty P, Byron K, Seymour JF, Szer J. Fludarabine-
based non-myeloablative chemotherapy followed by infusion of HLA-identical stem cells for relapsed leukaemia and lymphoma. Bone Marrow Transplant 1999;23:107-10.


27. Childs R, Clave E, Contentin N, Jayasekera D, Hensel N, Leitman S, et al. Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full do-
