Silver Spring, MD, USA). Optimas runs on a Pentium II computer system.

The identified reacting peptides were colored in a three-dimensional model of whole mouse ICAM-1, 1ic1.pdb, which was derived from the PDB-database [40] and was visualized using the software package Swiss PDB-viewer 3.7b1.
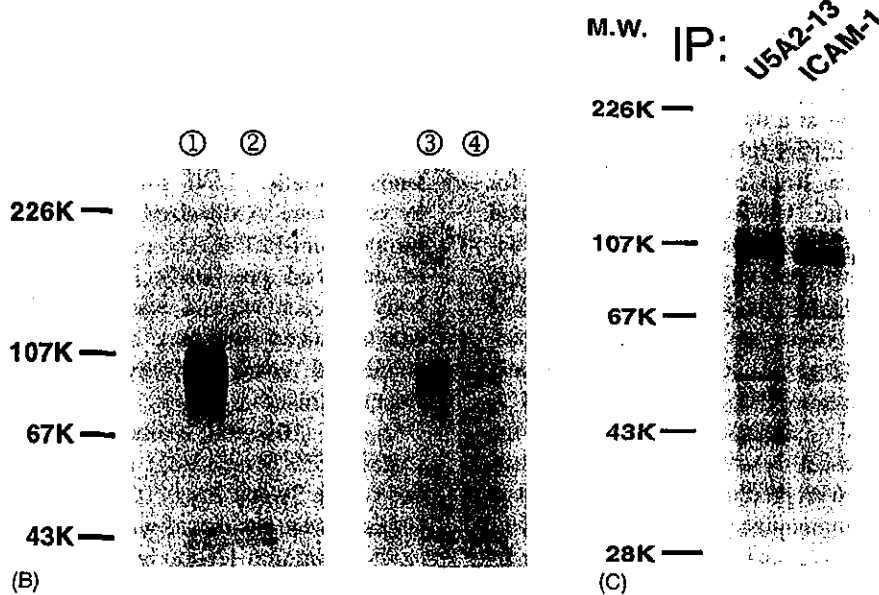
3. Results

3.1. Molecular cloning of a full-length cDNA encoding the antigen recognized by U5A2-13 mAb

We applied an expressional cloning strategy using COS-7 cells to isolate the gene encoding the antigen recognized
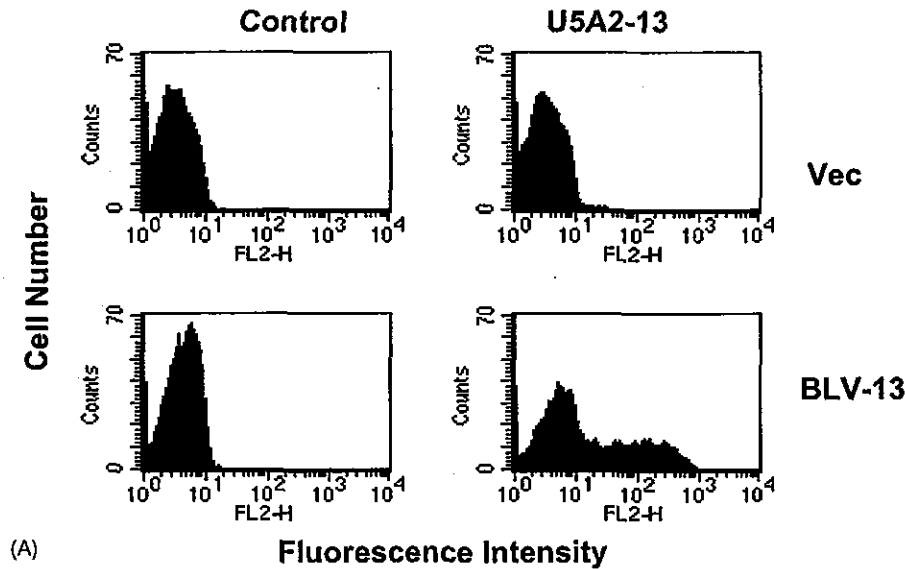
Fig. 1. Surface expression and biochemical analysis of U5A2-13-reactive molecules in COS-7 cells transfected with clone BLV-13 cDNA. (A) U5A2-13 mAb binds to COS-7 cells transfected with clone BLV-13 cDNA. COS-7 cells were transfected with pME18S vector alone (Vec) or with clone BLV-13 cDNA in pME18S (BLV-13), as indicated, right. Transfectants were stained with either PE-conjugated U5A2-13 mAb (U5A2-13) or PE-conjugated isotype-matched control Ab (Control) as indicated, above panel. Ab binding was detected by flow cytometry. (B) Western blotting of COS-7 cells transfected with clone BLV-13. Whole lysates of COS-7 cells transfected with clone BLV-13 were Western blotted. Lanes 1 and 2, transfected and control COS-7 cells detected with anti-ICAM-1 Ab, respectively; lanes 3 and 4, transfected and control COS-7 cells stained with anti-ICAM-1 Ab plus its blocking peptides, respectively. (C) Immunoprecipitation analysis of protein encoded by clone BLV-13 cDNA. Cells were labeled with biotin, then proteins were immunoprecipitated with U5A2-13 mAb and anti-ICAM-1 Ab. Immunoprecipitates were denatured with SDS sample buffer containing 2-ME and resolved in 7.5% SDS-PAGE gels and blotted. The blot was visualized using avidin-biotinylated horseradish peroxidase macromolecular complex and ECL® detection reagents.

by U5A2-13 mAb. A cDNA library was generated from murine BCL1 cells that were positive for U5A2-13 mAb using a pME18S expression vector. Not only NKT cells, but also some murine leukemia cell lines including BCL1, react with U5A2-13 mAb, but BCL1 expresses the most antigen (data not shown). A transfectant of a 2.8 kb cDNA clone, termed BLV-13, that was selected after two cycles of enrichment bound U5A2-13 mAb but not isotype-matched control IgG (Fig. 1A). Before sequencing this cDNA clone, we confirmed its enrichment in the second-round cDNA library by Southern blotting. Sequence analysis demonstrated that BLV-13 cDNA consisted of 2574 bp, encoding a polypeptide with 537 amino acid residues. A GenBank search revealed that the deduced amino acid sequence of BLV-13 was identical to mouse ICAM-1 (GenBank accession no. P13597).

We then Western blotted and immunoprecipitated lysates from BLV-13-transfected COS-7 cells using U5A2-13 and anti-ICAM-1 Abs. A band stained by anti-ICAM-1 Ab in the lysates of BLV-13-transfected COS-7 cells was dimin-

ished by its blocking peptides (Fig. 1B). Immunoprecipitates obtained from BLV-13-transfected COS-7 cells using U5A2-13 and anti-ICAM-1 Abs were detected as a 100 kDa protein band that corresponded to the molecular mass of mouse ICAM-1 (Fig. 1C). To confirm that U5A2-13 mAb reacts with mouse ICAM-1, hepatic mononuclear cells isolated from C57BL/6 mice and ICAM-1 deficient mice were dual-stained with anti-CD3 and one of U5A2-13, anti-ICAM-1 (clone 3E2) or anti-NK1.1 mAbs. We examined two strains of ICAM-1 mutant (exons 4 and 5) mice. U5A2-13 mAb was negative in both strains of ICAM-1 gene targeted mice (Fig. 2). These data demonstrated that our U5A2-13 mAb recognizes ICAM-1.

Furthermore, we sequenced 500 clones obtained from the cDNA library constructed after two cycles of enrichment (data not shown). One-hundred-and-twenty-three out of 500 clones had precisely the same cDNA insert as the 2.8 kb BLV-13 clone, suggesting the absence of an alternative splicing variant other than ICAM-1 mRNA encoding a specific protein recognized by U5A2-13 mAb. No other
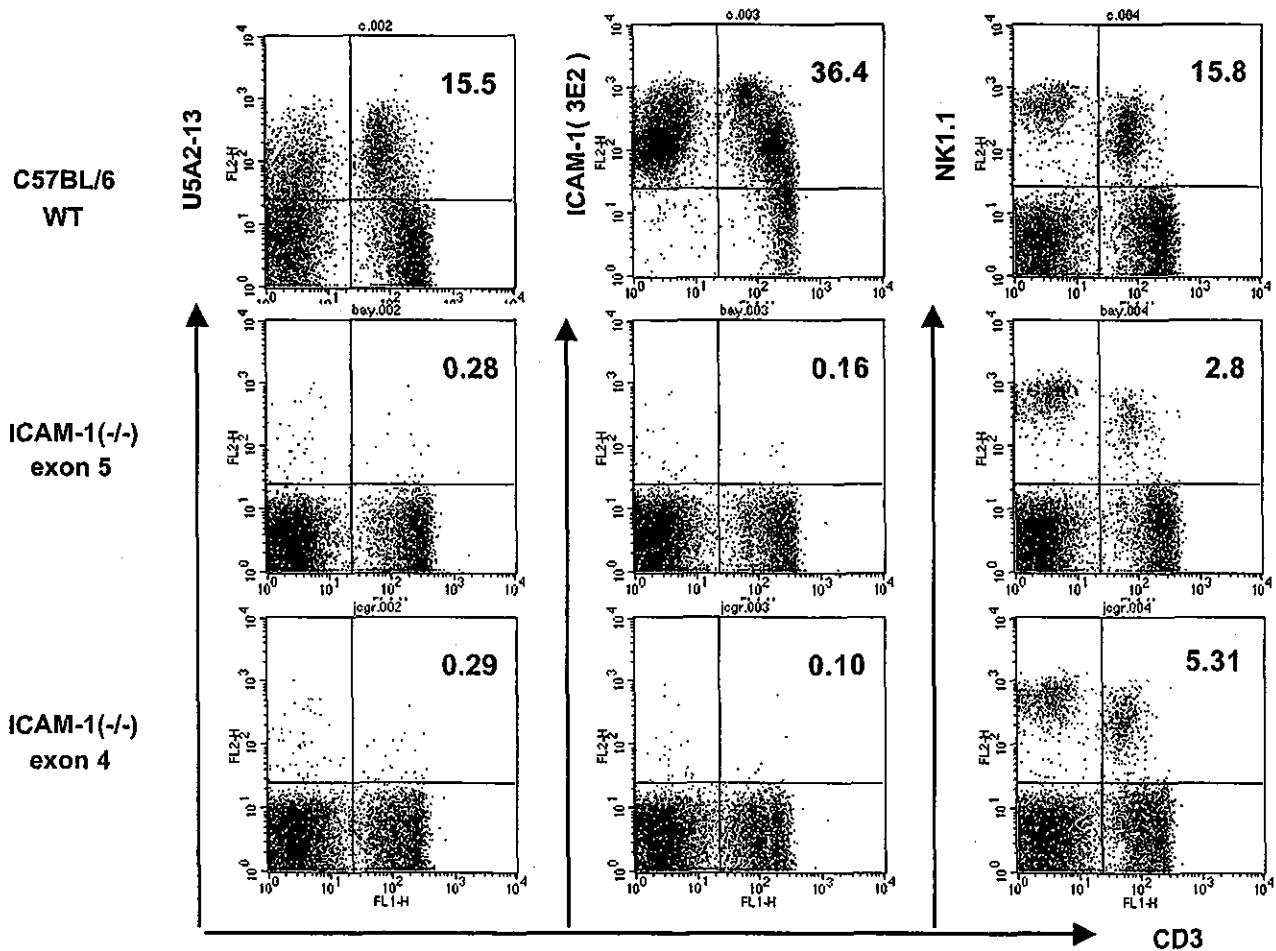

Fig. 2. U5A2-13 mAb did not react with lymphocytes in ICAM-1 deficient mice. Fresh hepatic mononuclear cells were isolated from C57BL/6 and two strains of mice deficient in ICAM-1 (exon 4 mutant and exon 5 mutant) then dual-stained with anti-CD3 mAb and one of U5A2-13 mAb, anti-ICAM-1 (clone 3E2) mAb, or anti-NK1.1 mAb. Data are representative of five individual experiments with similar results.
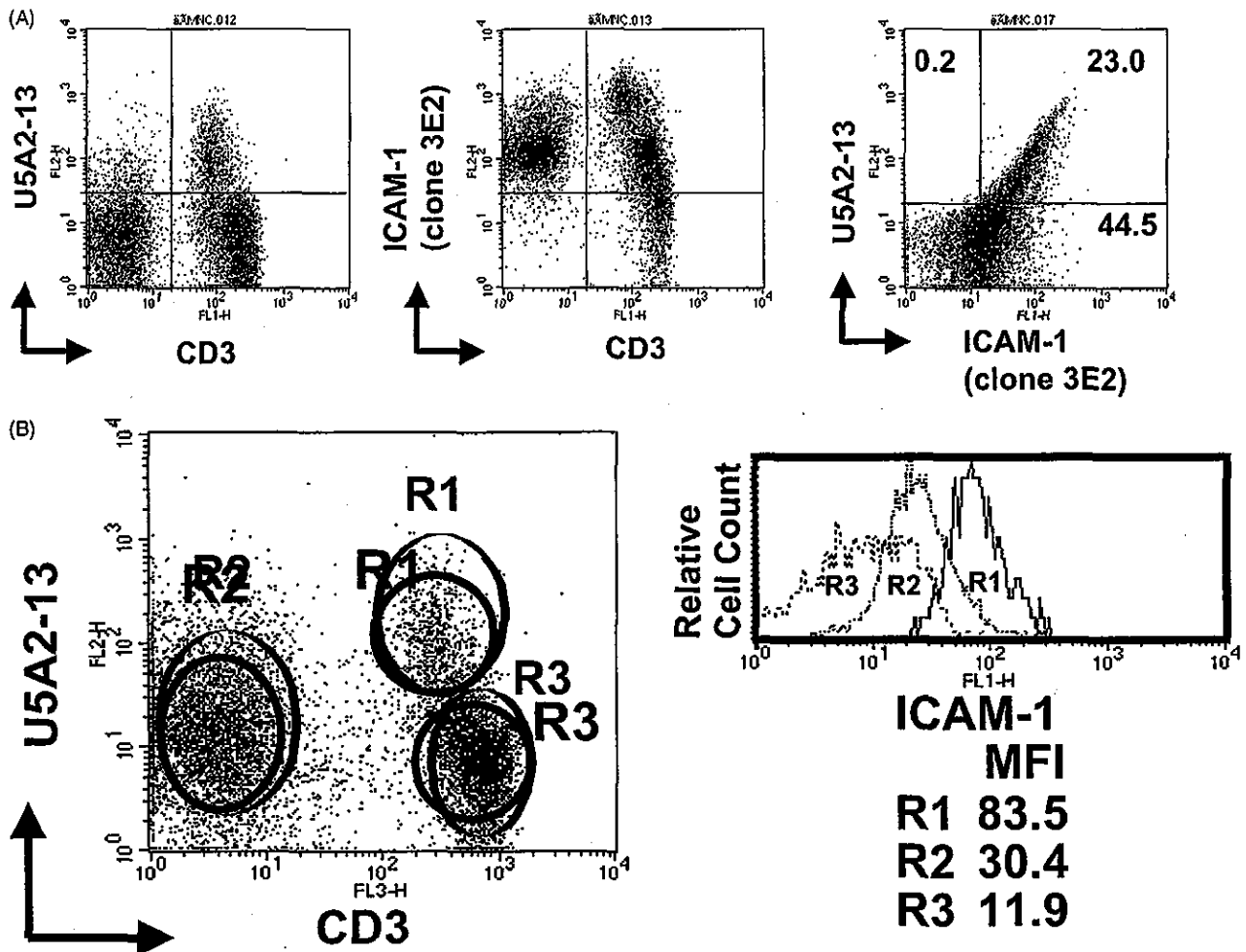
Fig. 3. Flow cytometry revealed that U5A2-13^{+} T cells correspond to ICAM-1^{high} T cells. Fresh hepatic mononuclear cells were isolated from C57BL/6 and stained with anti-CD3 mAb, U5A2-13 mAb and anti-ICAM-1 (clone 3E2) mAb (A). Mean fluorescence intensity (MFI) of ICAM-1 for each gated region is indicated below the panel (B). Data are representative of three individual experiments with similar results.

genes encoding a cell surface protein were found among the remaining 377 clones.

3.2. NKT cells express an epitope recognized by U5A2-13 mAb in extracellular domain two of ICAM-1

The profiles of hepatic mononuclear cells dual-stained with anti-CD3 mAb and one of U5A2-13, or anti-ICAM-1 (clone 3E2) mAbs were quite different (Fig. 3A). Three-color flow cytometry demonstrated that the U5A2-13 positive population encompasses CD3 positive, ICAM-1 (3E2) bright subsets, corresponding to approximately 35% of the 3E2 positive population (Fig. 3B). Flow cytometry revealed that the mean florescence intensity of ICAM-1 is very high not only murine, but also in human TCR Vα24 positive NKT cells (unpublished data).

We examined whether or not the epitopes of the antibodies are different, by performing a competitive inhibition assay. U5A2-13 mAb staining was not blocked by a prior

incubation with other anti-ICAM-1 mAbs such as clone 3E2, KAT-1, or YN1/1 (data not shown).

In addition, in order to determine U5A2-13 epitope, we generated synthetic peptides that represent amino acid sequences of ICAM-1 and examined their reactivity with U5A2-13 mAb by ELISA. Synthetic rod-attached peptides were used to map the antigenic sites on the extracellular domain of the ICAM-1. By means of the Pepscan method, all possible 2230 overlapping 30-mer peptides, composed of two 15-mer parts derived from the primary sequence, were synthesized. U5A2-13 mAb was tested against the synthetic minicard-peptides. Peptides were considered to represent antigenic sites if peaks occurred in a set of neighboring peptides and if at least one of the peaks in such a set amounted to more than three times the background. U5A2-13 mAb recognized a discontinuous epitope that was made of three parts of extracellular domain two of ICAM-1. The identified sites are: 161–178 region in B–C loop, 135–151 region in C′–E loop, and 188–204 region in F–G loop (Fig. 4).

Domain 1

Domain 2

SAVLLRGEELSRQPVGGHPKDPKEIFFTVLASRGD   B-C
HVDGGAPRTQLSAVLLR   C'-E
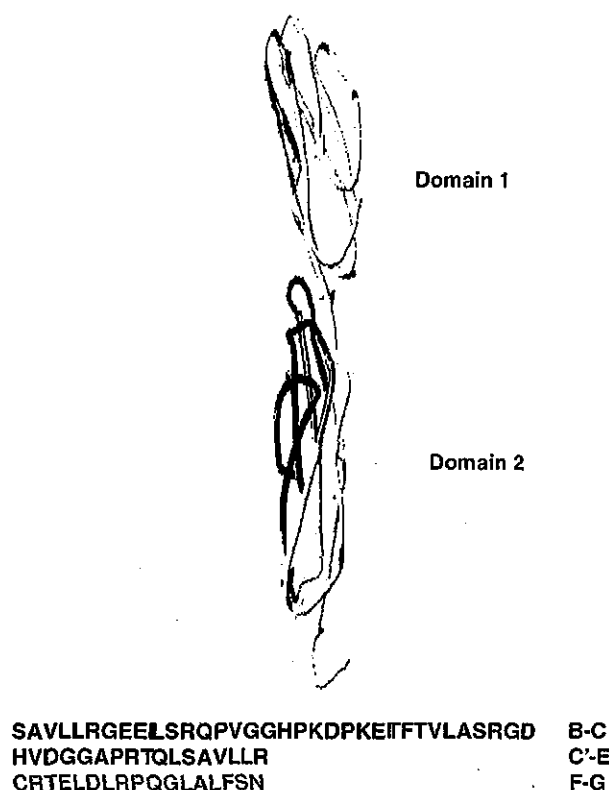CRTELDLRPQGLALFSN   F-G

Fig. 4. U5A2-13 epitope in a 3D model of human ICAM-1. U5A2-13 epitope consists of three loops in domain two of ICAM-1. The three homologous regions are demonstrated in a model of human ICAM-1(GenBank accession no. MMDB 8008). The core epitope is depicted in red. Two adjacent loops in blue and green are also part of the epitope.

Collectively, these data suggest that NKT cells express a unique epitope of ICAM-1 recognized by U5A2-13 mAb.

4. Discussion

Our studies demonstrated that an NKT cell surface antigen recognized by a novel mAb, U5A2-13, is encoded by *ICAM-1* gene and that its discontinuous epitope is composed of three loops located in extracellular domain two of ICAM-1. Expression of the classical NK marker, NK1.1 antigen, is confined to C57BL/6 and related strains, which hampers investigation in disease models of NK1.1-negative strains [1,2]. We showed previously that U5A2-13$^+$ T cells encompass a population functionally similar to NK1.1$^+$ T cells in various mouse strains such as C57BL/6, BALB/c, and C3H/He [28,30,31]. Furthermore, staining with U5A2-13 mAb yielded higher signal intensity for NKT cells than NK cells in FACS® analyses. This is unique compared with antibodies of other NK markers such as NK1.1, IL-2Rβ, Ly49 or DX5.

Recently the antigen recognized by DX5 has been molecularly cloned, which revealed CD49b (Very Late Antigen-2) [41]. Like U5A2-13, a surrogate NK marker, DX5, also corresponds to an adhesion molecule. This is not fortuitous but rather suggests that the importance of adhesion molecules

cannot be over-emphasized in NK and NKT cell function. NKT cells are considered to activate NK cells rapidly in the innate immune system [9]. NK cells express large amount of LFA-1 [42]. Our study showed that NKT cells express ICAM-1 most. This constitutes a reasonable evidence for efficient cross-talk between these cells.

The staining profiles of hepatic MNCs with U5A2-13 mAb and with other conventional anti-ICAM-1 mAbs (such as 3E2, YN1/1 or KAT-1) were quite different (Fig. 3A). U5A2-13 epitope resides in domain two. The epitopes of the other anti-ICAM-1 antibodies, on the other hand, are located in domain one, which is the site responsible for binding with its ligand LFA-1. Therefore, in competitive inhibition assay, staining with U5A2-13 mAb was not blocked by these antibodies (unpublished observation). We cannot exclude a possibility that, because of its affinity for ICAM-1, U5A2-13 mAb recognizes only the ICAM-1^{high} population when ICAM-1 is present in substantial amounts on the cell surface. Another possibility is that freshly isolated naïve NKT cells express a unique epitope in domain two of ICAM-1 in such a manner that it can efficiently react with U5A2-13 mAb. The ICAM-1 epitope recognized by U5A2-13 mAb may depend on glycosylation selective to NK cells. Accumulating evidence shows that ICAM-1 forms homodimers on the cell surface that are more efficient than monomers in binding to its ligand, LFA-1 [43–46]. In freshly harvested mononuclear cells, staining with U5A2-13 mAb is mostly confined to NKT cells but not to conventional T and B cells. We also revealed that, upon activation by a mitogen, not only NKT cells but also T and B cells start to react with U5A2-13 mAb [47]. Taken together, these findings may indicate that homodimers of ICAM-1 on the surface of activated ICAM-1^{high} cells form an epitope for U5A2-13 mAb. ICAM-1 per se is widely expressed on lymphocytes but it is not until they get fully activated and become ICAM-1^{high} when they begin to show U5A2-13 epitope otherwise concealed. Similarly, NK1.1 antigen is also induced on CD8$^+$ T cells upon activation [48].

It is of interest that this U5A2-13 epitope on NKT cells is related to their recognition and functions. In vivo administration of U5A2-13 mAb not only modulates cytokines production by NKT cells upon stimulation with α-GalCer but also increases tumor metastases in a B16 melanoma model (manuscript in preparation). We conclude that NKT cells express a unique U5A2-13 epitope of ICAM-1.

Acknowledgements

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Infections post transplant

A nationwide survey of deep fungal infections and fungal prophylaxis after hematopoietic stem cell transplantation in Japan

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Summary:

We conducted a nationwide survey to define incidence of deep fungal infections and fungal prophylaxis practices after HSCT. In all, 63 institutions responded. Total number of in-patient transplantations was 935: 367 autologous, 414 allogeneic myeloablative, and 154 allogeneic reduced-intensity (RIST) ($n = 154$). Number of patients who were cared for in a clean room at transplant was 261 (71%) in autologous, 409 (99%) in conventional and 93 (66%) in RIST, respectively. All patients received prophylactic antifungal agents; 89% fluconazole. Number of patients who received the dosage recommended in the CDC guidelines (400 mg/day) was 135 (42%) in conventional transplant and 34 (30%) in RIST ($P = 0.037$). Number of patients who received fluconazole until engraftment and beyond day 75 in conventional transplant vs RIST was, respectively, 324 (100%) vs 109 (97%), and 39 (12%) vs 18 (16%), with no significant difference between the two groups. A total of 37 patients (4.0%) were diagnosed with deep fungal infections; autologous transplantation (0.03%), conventional transplantation (6.0%) and RIST (7.1%). Wide variations in antifungal prophylaxis practice according to the type of transplant and the institutions, and deep fungal infection remain significant problems in RIST.
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Fungal infection is a common complication after hematopoietic stem cell transplantation (HSCT), and the primary causative organisms are *Candida* and *Aspergillus*, with significant mortalities even if properly treated with antifungal agents.[1] Therefore, antifungal prophylaxis has been emphasized following HSCT.[2] In 2000, the Centers for Disease Control and Prevention (CDC) in the United States issued guidelines for the prevention of fungal infections in the setting of allogeneic HSCT and some cases of autologous transplantation,[3] and these are considered a gold standard in many countries throughout the world, including Japan. To prevent *Candida* infections, the CDC guidelines recommend the use of fluconazole (400 mg/day) until engraftment,[3] based on the results of two randomized controlled studies published in 1992 and 1995.[4,5] However, since then, the circumstances surrounding HSCT have been changing rapidly. The use of fluconazole has been questioned, since it is ineffective against *Aspergillus* species. Moreover, the rationale for selecting the recommended dose of 400 mg/day fluconazole and the optimal duration of prophylaxis need to be clarified.[6] A major concern regarding emerging fluconazole-resistant *Candida* species needs to be critically evaluated.[7,8]

Aspergillus is the most common pathogen in fungal infections in the course of transplantation or treatment for leukemia.[9] The importance of hospital environment control has been emphasized for effective prevention. The outbreak

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of nosocomial infections due to the renovation of transplant buildings[10,11] and via the water system in transplant wards has been reported.[12] The CDC guidelines recommend the use of air conditioning systems/tools.[3] However, the guidelines may have become outdated due to changing circumstances. First, the timing of the development of aspergillosis has changed. Most *Aspergillus* infections occur late beyond 100 days after transplantation.[13] This trend may be enhanced by the wider application of reduced-intensity stem cell transplantation (RIST) on an outpatient basis.[14] Second, newly developed antifungal agents have been shown to be effective in the prevention of *Aspergillus* infection.[15,16] Moreover, a recent drastic change is that RIST has been extended to unrelated transplantation,[17] umbilical cord blood transplantation[18] and elderly patients.[19] RIST has the advantage of a shorter period of neutropenia, and it is likely that the incidence of *Candida* infection is reduced. On the other hand, the incidence of steroid-therapy requiring GVHD remains the same as in conventional transplantations, and the intensity of immune suppression is similar.[20] It is expected that late-occurring fungal infections by *Aspergillus*, etc may become a significant problem after RIST.[21–23]

Thus, a re-evaluation and update of the guidelines appears to be necessary, particularly in the area of RIST. There have been only a few studies on fungal infections after RIST.[21–23] The objective of this study was to survey antifungal prophylaxis in Japanese transplantation practice, and to compare the findings in RIST, conventional allogeneic transplantation (CST), and autologous transplantation.

Patients and methods

Data collection

Of the 418 Japanese medical institutions in which HSCT is performed, 122 institutions agreed to participate in this survey. Each of these institutions received a questionnaire that included the following items: the number of transplantations performed in 2001, the type of the patient's disease and the type of transplantation, use of a clean room or designated room for transplantation, practice regarding prophylactic use of antifungal agents, and occurrence of systemic fungal infections. For cases that developed deep fungal infections, age, sex, date of onset, diagnostic approaches, infected organs, pathogens, neutrophil count at onset, coexistence of GVHD, use of immunosuppressants, prophylactic use of antifungal agents, and patient outcome were also recorded.

We used the EORTC/NIH-MSG criteria for the diagnosis of deep fungal infections.[24] We defined both proven and probable infectious cases as deep fungal infection.

Antifungal prophylaxis measures

We defined the nine measures listed below as measures for preventing fungal infection. All of the methods were evaluated as A or B based on the evidence level in the

CDC guidelines, as previously reported.[25] The guidelines use a combination of category (A–D) and the recommendation (I–III). We used two recommendation levels, A and B, which indicated strong or moderate evidence supported by well-established clinical trials (I or II) or respected authorities (III).

(a) Yeast infection following allogeneic HSCT

(a1) Medical staff in contact with HSCT recipients should follow appropriate hand-washing practices to safeguard patients from exposure (Evidence level A III).

(a2) Fluconazole (400 mg/day, orally or intravenously) should be administered from the day of transplant until engraftment (Evidence level A II).

(b) Mold infections following allo-SCT

(b3) HSCT recipients who remain immunocompromised should avoid hospital construction or renovation areas (Evidence level A III).

(b4) Use of high efficiency particulate air (HEPA) filtration (Evidence level B III).

(b5) Air should flow from patient rooms to corridors (Evidence level B III).

(b6) Correctly sealed rooms, including correctly sealed windows and electrical outlets (Evidence level B III).

(b7) High rates of room air exchange (ie, >12 air changes/h) (Evidence level B III).

(b8) Barriers between patient care areas and renovation or construction areas to prevent dust from entering patient care areas. The barriers must be impermeable to Aspergillus species.

(c) Autologous HSCT

(c9) Autologous HSCT recipients generally are at lower risk of invasive fungal infection than allogeneic HSCT recipients. Autologous HSCT recipients do not require routine intense anti-yeast prophylaxis. Nevertheless, some researchers recommend the use of an anti-yeast prophylaxis in patients who have underlying hematologic malignancies, and who have or will have prolonged neutropenia and mucosal damage from intense conditioning regimens or graft manipulation, or who have recently received purine analogues (Evidence level B III). Regarding mold infections, no guideline has been reported for autologous HSCT.

Prophylaxis for deep fungal infections in Japan

In Japan, the antifungal agents approved for the treatment of deep fungal infections in 2001 included amphotericin B, fluconazole, itraconazole, miconazole and terbinafine. With the exception of itraconazole, they can be administered either orally or intravenously. The only available formula of itraconazole was a capsule, and in this form, absorption through the gastrointestinal tract is inconsistent.[26] Itraconazole oral solution, voriconazole, liposomal amphotericin B and echinocandin have not yet been approved.

Governmental approval has not yet been granted for the prophylactic use of any antifungal agents, although this is widely applied as a practice in most medical institutions.[27] Fluconazole, up to 400 mg, is approved only for the treatment of fungal infection.

Use of a clean room within a transplant ward is reimbursed by insurance. We considered that prophylactic measures (b3)–(b8) were satisfied if HSCT was performed in a clean room. A clean room was defined as an isolated room equipped with HEPA filtration and air flow toward an exit of the room with sealed windows.

End points and statistical methods

The objective of this study was to conduct a survey of antifungal prophylaxis in HSCT. The levels of compliance with the established prophylactic measures in RIST and CST were compared. The second objective was to compare the CST, RIST and auto-SCT groups with respect to the incidence and characteristics of fungal infections.

We examined (a2) and (b3)–(b8) of the nine items listed above. Item (a1) was excluded from the survey because of inconsistency of proper evaluation in a retrospective survey. We considered that items (b3)–(b8) were satisfied when a clean room was used. We aimed to evaluate differences in fungal prophylaxis between conventional and reduced-intensity transplants. We did not collect detailed information on transplantation procedures such as stem cell sources and drugs used in the preparative regimens. A univariate analysis using Fisher's exact test and the Mann–Whitney U test was performed to compare the differences in prophylactic measures between RIST and CST. Values of $P<0.05$ were considered significant.

Results

Patients background

We received questionnaires from 63 medical institutions, representing 935 transplantations. In Japan, a total of 1964 transplantations were performed in 2001,[28] and approximately half of the patients were surveyed in this study. The median number of transplantations per institute/year was 10 (range, 1–108). The types of transplantation were autologous HSCT (367, 40%), CST (414, 45%), and RIST (154, 15%). All of the RIST recipients received purine-analog-based preparative regimens with or without low-dose TBI.

Patients' diseases included malignant lymphoma (191 cases, 21%), acute myelocytic leukemia (147, 16%), acute lymphocytic leukemia (107, 11%), multiple myeloma (80, 9%), chronic myelocytic leukemia (74, 8%), myelodysplastic syndrome (51, 5%), solid tumors (50, 5%), aplastic anemia (16, 2%), and others (219, 23%).

All patients were hospitalized during transplantation. The number of autologous HSCT, CST and RIST recipients who were in a clean room at the time of transplantation was 261/367 (71%), 409/414 (99%) and 93/154 (66%), respectively.

Prophylactic use of antifungal agents

All patients including autologous HSCT cases received an antifungal agent as a prophylaxis, and azole antifungal agents were administered in 742 patients (79%). The most commonly used azole antifungal agent was fluconazole (89%). Figure 1 shows the types of agents used in CST, RIST and autologous HSCT.

Fluconazole was administered to 324 (78%) CST and 112 (73%) RIST recipients. The numbers of patients who received fluconazole 400, 200 and 100 mg/day were 135 (42%), 169 (52%) and 20 (6%), respectively. Those patients who were given fluconazole 400, 200 and 100 mg/day were 34 (30%), 70 (63%) and 8 (7%), respectively. Significantly larger doses of fluconazole were used in CST than in RIST ($P=0.037$).

The numbers of patients who received fluconazole until engraftment and beyond day 75 in the CST and RIST groups were, respectively, 324 (100%) and 39 (12%), and 109 (97%) and 18 (16%). Duration of fluconazole use was not significantly different between CST and RIST.

Incidence and clinical characteristics of deep fungal infections

Of the 935 transplant cases, 37 (4.0%) were diagnosed with deep fungal infections (13 proven and 24 probable cases); 0.03% (1/367) of autologous HSCT, 6.0% (25/414) of CST, and 7.1% (11/154) of RIST. The causative organisms included Candida ($n=9$), Aspergillus ($n=16$), Mucor ($n=1$), Fusarium ($n=1$) and unknown ($n=10$). The median onset date from the time of transplant was 85 days (range, 1–392 days): 92 days in CST and 117 days in RIST, with nine cases developing within 30 days of transplant (one case in autologous HSCT, seven in CST and one in RIST). Three cases of 37 deep fungal infections had a previous history of fungal infection. All of them received fluconazole and AMPH-B intravenously as a prophylaxis.
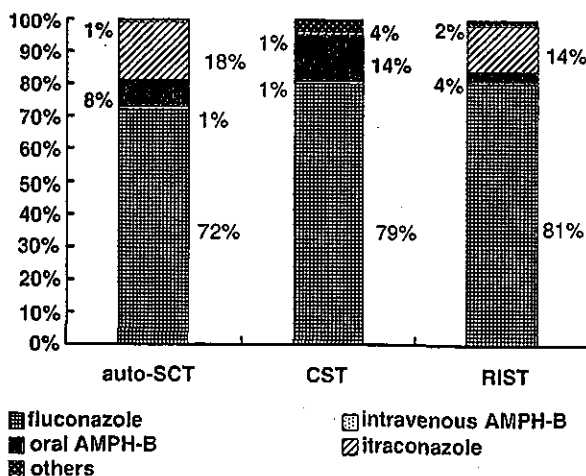

Figure 1 Types of prophylactic antifungal agents used in each type of HSCT Azole agents were the most-administered drugs for prophylactic use in HSCT patients.

The causative organisms for the nine cases of candidiasis included *C. albicans* ($n = 4$), *C. glabrata* ($n = 2$), *C. tropicalis* ($n = 1$), *C. inconspicua* ($n = 1$), and a combination of *C. lusitaniae* and *C. guilliermondii* ($n = 1$). The infected organs included blood (fungemia, six cases), the respiratory system ($n = 2$) and the liver and spleen ($n = 1$). Among four patients who were infected with *C. albicans*, two had not received any antifungal agents including fluconazole as a prophylaxis, while the remaining two cases had been on fluconazole prophylaxis and were considered to be prophylaxis failure. Among these four patients, three started or continued to receive fluconazole following infection, and three patients subsequently died.

The causative organisms for the 16 cases of aspergillosis included *Aspergillus* spp. ($n = 14$), *A. fumigatus* ($n = 1$) and *A. terreus* ($n = 1$). Six and 10 of these patients received RIST and CST, respectively. In all, 14 and two patients had received prophylactic fluconazole and itraconazole, respectively. GVHD requiring corticosteroid therapy was documented at the onset of the disease in 13 of the 16 cases. The initially infected organ was the respiratory system in all cases, with a median onset of invasive aspergillosis of 85 days (range, 9–392). Four of the 16 patients were neutropenic at the diagnosis of aspergillosis. Treatment for invasive aspergillosis included intravenous AMPH-B (13 cases), oral itraconazole (two cases), and fluconazole (one case). Eight patients subsequently died without resolution of invasive aspergillosis.

Comparison of CST and RIST recipients

Table 1 shows the characteristics of fungal infections developed in CST and RIST recipients. There was no significant difference between the two groups with respect to the incidence, date of onset or death rate.

Discussion

This study highlights the current problems in antifungal prophylaxis. In Japan, a total of 1964 transplantations were performed in 2001,[28] and about half of these were surveyed in this study. We believe that sufficient data were collected to characterize prophylaxis practice and fungal infection in this country, while it is limited by bias as a retrospective design, and we should be careful in interpreting the results of this study.

With improvements in prophylactic measures, the incidence of early mold infections has decreased,[14,29,30]

and most infections occur late in association with GVHD.[13] The need for a HEPA filter or laminar air flow (LAF) may be questioned in RIST. All transplant wards in Japan, but not all nontransplant wards, are equipped with these systems. Although 99% of CST was performed in transplant wards, this percentage decreased to 71% in autografts and 66% in RIST. The potential economic benefits of RIST may be related to an increase in RIST performed in nontransplant wards without expensive air filtration systems.

In autologous HSCT, the prophylactic use of antifungal agents is recommended only for high-risk patients[3] based on several clinical studies.[4,31] However, this study demonstrates that all of the Japanese autologous HSCT recipients received some prophylactic antifungal agents. This is an overuse of antifungal agents because the overall risk of fungal infection in autologous transplants is lower than allogeneic transplant. There are several possible explanations for this overuse. Since the definition of 'low-risk patients' in the CDC guidelines is unclear, physicians take a more conservative way to reduce the risk of fungal infection. It has been suggested that long-term use of azole antifungal agents may cause resistance.[32] Additionally, azole antifungal agents are expensive, and long-term use is not economical.[33] On the other hand, for the prophylactic use of antifungal agents in allogeneic transplant, the CDC recommends prophylactic fluconazole 400 mg/day during the neutropenic period following transplantation. However, the rationale for the selection of fluconazole from among many other available antifungal agents, its dose setting and prophylaxis period remain unclear. Its usefulness in RIST recipients has not been established.

After the CDC issued its guidelines in 2000, various drugs, including voriconazole, itraconazole (oral solution and intravenous formulation), and echinocandin, have been developed and are now commercially available. Moreover, *Aspergillus* is not susceptible to fluconazole, and recent comparative studies suggest that itraconazole is more effective than fluconazole in the prevention of *Aspergillus* infection.[15,34] Hence, the recommendation of fluconazole over any other agent may no longer be defensible. Nevertheless, this study clearly documented that fluconazole was used in 75% of allogeneic transplant in Japan. This bias is likely due to the CDC recommendation and to the fact that an alternative antifungal agent is not commercially available in Japan. As of June 2003, the only available itraconazole formula in Japan is a capsule, which carries an obvious risk of inadequate

Table 1   Comparison on clinical features of deep fungal infection between CST and RIST

		CST (n = 414)	RIST (n = 154)
Number of deep fungal infection		26 (6.3%)	11 (7.1%)
Median onset (days after transplant) (range)		77 (1–392)	117 (28–182)
Presence of GVHD at the onset of fungal infection	Present/absent	12/14	9/2
Presence of neutropenia at the onset of fugal infection[a]	Present/absent	8/18	1/10
Use of corticosteroid at the onset of fungal infection	Yes/no	15/11	7/4
Causative organisms	Aspergillus/Candida/Others	17/7/2	9/1/1
Mortality		81%	82%

[a]Neutropenia was defined as neutrophils below 0.5×10^9/l.

absorption of the drug. Voriconazole and caspofungin have not yet been approved, and micafungin was approved only recently. Regarding *Aspergillus* infections, it is difficult to conclude that the CDC's recommendation for the prophylactic administration of fluconazole is useful. With .the present availability of all of these alternative agents, a comparative study to identify a suitable procedure will be required.

While all of the patients received prophylactic fluconazole at least until engraftment, only 44% received the recommended dose of 400 mg/day. Moreover, only 20% of both the CST and RIST recipients received fluconazole beyond 75 days following transplantation, as recommended in a previous study.[6] It has been reported that *C. albicans* can be controlled at a lower dose of 200 mg/day.[35,36] Many physicians believe that 400 mg of fluconazole is not required for prophylactic use, and optimal duration of fluconazole prophylaxis remains to be established. Since fluconazole is expensive and costs about 100 000 yen ($850) when used at 400 mg/day to cover from the commencement of pre-transplant treatment and engraftment, validation of the adequate dose and the duration for prophylactic use is important.

With an increasing number of patients undergoing transplant, establishment of fungal management is important in RIST. The practice for the prevention and treatment of fungal infection varies among institutions. Mortality of invasive aspergillosis was 50% in this survey, which were far lower than reported previously.[1] The differences might be attributable to diagnostic approaches. In Japan, diagnostic measures using computed tomography and blood tests such as beta-D-glucan assay or an enzyme-linked immunosorbent test detecting galactomannan antigen are widely used.[37–39] These tests might have contributed to make an early diagnosis of aspergillosis, improving its prognosis. These situations are similar to antifungal prophylaxis. The guidelines for antifungal prophylaxis, which were prepared based on previous clinical studies, should be updated, since the circumstances surrounding transplantation have been changing. However, there are little data to make new recommendations for guidelines of antifungal prophylaxis, and more information is needed regarding fungal infections following RIST. Further investigation is needed to determine what measures are effective to accommodate the changes in transplant practices.

Acknowledgements

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Appendix

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bjh research paper

Comparative analysis of clinical outcomes after allogeneic bone marrow transplantation *versus* peripheral blood stem cell transplantation from a related donor in Japanese patients

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Summary

A reduced incidence of graft *versus* host disease (GvHD) has been documented among Japanese allogeneic bone marrow transplantation (BMT) patients, as the Japanese are genetically more homogeneous than western populations. To clarify whether this ethnic difference affects the results of allogeneic peripheral blood stem cell transplantation (PBSCT), we conducted a nationwide survey to compare clinical outcomes of allogeneic PBSCT ($n = 214$) and BMT ($n = 295$) from a human leucocyte antigen-identical-related donor in Japanese patients. The cumulative incidence of grades II–IV acute GvHD was 37·4% for PBSCT and 32·0% for BMT. The cumulative incidence of extensive chronic GvHD at 1 year was significantly higher after PBSCT than BMT (42% vs. 27%; $P < 0·01$). The organ involvement patterns of GvHD were different between the two groups. By multivariate analyses, the incidence of chronic GvHD was significantly increased in PBSCT, whereas the stem cell source did not affect the incidence of acute GvHD, transplant-related mortality, relapse or survival. We concluded that Japanese PBSCT patients have an increased risk of chronic GvHD compared with BMT patients, but the incidence of acute GvHD was still lower than in western populations. Thus, the choice of haematopoietic stem cell source should be considered based on data for individual ethnic populations.

Keywords: Japanese, marrow transplantation, stem cell transplantation, graft *versus* host disease.

During the past decade, peripheral blood stem cell transplantation (PBSCT) has been explored in the autologous as well as the allogeneic haematopoietic stem cell transplantation (HSCT) setting as an alternative to bone marrow transplantation (BMT). Although there were some inconsistencies in the early reports, it appears that haematological recovery is faster, but the incidence of acute graft *versus* host disease (GvHD) is similar, and chronic GvHD is more frequent in allogeneic PBSCT patients than in BMT patients (Schmitz *et al*, 1998, 2002; Blaise *et al*, 2000; Champlin *et al*, 2000; Heldal *et al*, 2000; Powles *et al*, 2000; Bensinger *et al*, 2001; Cutler *et al*, 2001; Couban *et al*, 2002; Ringden *et al*, 2002). Additionally, some investigators have reported improved survival after PBSCT compared with BMT (Powles *et al*, 2000; Bensinger *et al*, 2001; Couban *et al*, 2002).

Although a number of small prospective randomized-controlled trials (RCTs) have been published, cautious interpretation is required because the primary end points of these studies were safety (Schmitz *et al*, 1998), engraftment (Blaise *et al*, 2000; Heldal *et al*, 2000; Powles *et al*, 2000) and equivalency of acute GvHD (Bensinger *et al*, 2001). Because of the small sample size in these studies, the statistical power was not enough to detect differences in important, clinically relevant outcomes between PBSCT and BMT, such as chronic GvHD, relapse rate, transplant-related mortality (TRM) and survival. In an attempt to clarify this, several large RCTs and meta-analyses have recently been published (Cutler *et al*, 2001; Couban *et al*, 2002; Schmitz *et al*, 2002; Horan *et al*, 2003).

However, findings in western populations cannot be directly transferred to other ethnic populations, where the incidence of GvHD differs. Most previous studies that compared BMT and PBSCT were from western countries. While detailed information on the ethnics of the study population was not provided, most patients would have been Caucasian. In Japanese BMT patients, the incidence of acute GvHD is considered to be lower than in western countries because of the relative genetic homogeneity of the population (Morishima *et al*, 1989; Oh *et al*, 2002; Lin *et al*, 2003). Whether this ethnic difference also affects the results of PBSCT, as reflected in differences in the incidence of GvHD, relapse and survival, has not been established. Apart from the intense eradication of malignant cells by the conditioning regimen, the main therapeutic benefit of allogeneic HSCT relies on the induction of immune-mediated graft *versus* leukaemia (GVL) effect (Horowitz *et al*, 1990). This GVL effect may also have a different impact in different ethnic groups. Therefore, to survey outcomes after allogeneic HSCT in Japan, we conducted a retrospective, multi-centre study comparing allogeneic PBSCT with BMT from a human leucocyte antigen (HLA)-identical related donor in 509 patients with leukaemia or myelodysplastic syndrome (MDS). We also aimed to determine the impact of GvHD on relapse and survival after transplantation.

Patients and methods

Methods

Transplantation centres across Japan were contacted and asked to provide data on all consecutive allogeneic HSCT from a family donor using report forms with specific addenda. Recipients of T-cell-depleted blood stem cell transplants, those receiving reduced-intensity stem cell transplantation, and those who had received bone marrow together with PBSC were not reported. Between January 1999 and October 2001, a total of 629 adult patients with leukaemia or MDS received a myeloablative preparative regimen and allogeneic BMT or PBSCT from an HLA-identical-related donor (matched at HLA-A, -B, -DR by serological or molecular testing) in 82 participating centres (Appendix A). Patients who did not receive GvHD prophylaxis using ciclosporin A (CsA) and methotrexate (MTX) ($n = 41$), those who did not receive granulocyte colony-stimulating factor (G-CSF) postallograft ($n = 75$), those who had undergone autografting previously ($n = 3$) and those who had double cancer ($n = 3$) were excluded. Finally, a total of 509 patients were included in this analysis. The stem cell source was decided according to the protocol of each transplantation centre. The medical records were reviewed retrospectively for patients' demographic data, date of engraftment, onset of acute and chronic GvHD, grading and organ involvement from the date of transplantation to the date of death or last contact. Computerized error checks and physician review of submitted data were performed to ensure data quality.

End point definitions

End points were assessed on the date of last patient contact and were analysed as of 31 May 2002. The study focused on haematopoietic recovery, acute and chronic GvHD, target organs of GvHD, TRM, progression-free survival (PFS) and overall survival (OS) after PBSCT compared with BMT. The day of neutrophil engraftment was defined as the first of three consecutive days on which the patient's absolute neutrophil count was above $0.5 \times 10^9/l$. The day of platelet engraftment was defined as the first of seven consecutive days on which the platelet count was above $20 \times 10^9/l$ without platelet transfusion. Engraftment failure was diagnosed as when engraftment was not achieved at any time after transplantation. The diagnosis of GvHD was based on clinical evidence with histological confirmation whenever possible. Acute GvHD within the first 100 d after transplantation was graded according to standard criteria by attending physicians of each hospital (Przepiorka *et al*, 1995). Patients who survived at least 100 d without relapse or disease progression, with sustained donor engraftment, were evaluated for chronic GvHD. Chronic GvHD was graded as limited (localized skin or single organ involvement) or clinically extensive (Shulman *et al*, 1980).

Patients without GvHD were censored at the time of relapse, disease progression, death or last follow-up. GvHD after donor leucocyte infusion was not included in this analysis.

Standard risk diseases were defined as acute myeloid leukaemia (AML) or acute lymphoblastic leukaemia (ALL) in first remission; chronic myeloid leukaemia (CML) in chronic phase; and refractory anaemia without excess of blasts (Bensinger *et al*, 2001). All other stages of these diseases and all other types of leukaemia were considered as high risk. The Eastern Cooperative Oncology Group (ECOG) scale was used to evaluate performance status (PS) at the time of transplantation. PFS was measured as the time from the day of transplantation until disease relapse or progression, death from any cause or second transplantation for graft failure or rejection. Both relapse and progression were defined as disease progression with TRM being censored. TRM included all causes of death other than disease progression or relapse occurring at any time after transplantation. Reported causes of death were reviewed and categorized. Patients who died as a result of relapse or disease progression after transplantation were considered to have died of their original disease. Similarly, patients who died of active GvHD were considered to have died of this complication even if other complications (e.g. infection) were recorded as the proximate cause. All deaths were considered for estimating the OS.

Statistical analysis

The primary end point of the comparison was the cumulative incidence of acute and chronic GvHD. The secondary end points included the incidence of relapse, TRM, PFS and OS. The following patient or transplant characteristics were analysed for their prognostic value on each of the outcomes: patient and donor age (less than or more than 40 years), sex, sex matching, ECOG PS, disease risk, cytomegalovirus serology, stem cell source, conditioning regimen and doses of MTX. To compare the two groups of patients receiving PBSC or BM, we used the chi-square test for categorical variables and the non-parametric Mann–Whitney *U*-test for ordered categorical and continuous variables. The unadjusted probabilities of PFS and OS were estimated from the time of transplantation using the Kaplan–Meier product limit method, according to the risk group, and 95% confidence intervals (CIs) were calculated using the Greenwood formula (Kaplan & Meier, 1958). To compare these two outcomes between the graft types, the log-rank test was used. In calculating the time-to-event for analysis of neutrophil/platelet engraftment, acute/chronic GvHD, TRM or relapse where competing risks alter the assessment of frequency, cumulative incidences were estimated (Gooley *et al*, 1999).

Association of graft type and each of the outcomes were mainly evaluated with multivariate Cox proportional hazards models (Cox, 1972). The occurrence of acute and/or chronic GvHD was included as a time-dependent covariate. The proportional hazards assumption of the Cox model was assessed mainly by a graphical approach. To confirm the results concerning the effects of graft type obtained from Cox analyses, we also presented results that adjusted the baseline confounding by the inverse probability-of-treatment weighted (IPTW) method (Robins *et al*, 2000). This method is less restrictive than the Cox model because we did not need to correctly specify any assumption between time to each event and baseline factors. We modelled the probability that a patient received PBSCT using the logistic regression with all the baseline factors described above as explanatory variables. From this logistic regression model, estimates of the patient specific weight, i.e. the inverse of the conditional probability of receiving his/her own graft type, were obtained. The subject-specific weight was used to estimate the effect of graft type. This weight is the probability that a subject would have his/her own observed transplantation. For IPTW estimates, the conservative robust variance estimates were used to construct confidence intervals (Lin & Wei, 1989). For end points other than relapse, cumulative incidence functions were predicted from the proportional (subdistribution) hazards model (Fine & Gray, 1999) and adjusted for effects of significant covariates in the multivariate Cox models explained above. The weights were the sample population value for each prognostic factor. SAS version 8.2 (SAS Institute Inc., Cary, NC, USA) and S Plus 2000 (Mathsoft, Seattle, WA, USA) were used for all statistical analyses.

Results

Patient and transplantation characteristics

Patient and transplantation characteristics are summarized in Table I; 295 patients received BMT and 214 received PBSCT. Regarding the diagnosis of their disease, 188 (36·9%) had AML, 144 (28·3%) had CML, 108 (21·2%) had ALL, 50 (9·8%) had MDS, and 19 (3·7%) had other types of leukaemia. The standard risk disease cohort consisted of 307 patients (60·3%), and the remaining 202 (39·7%) were of high-risk disease status. Conditioning before transplantation was a total body irradiation (TBI)-based regimen (74·9% in BMT, 64·5% in PBSCT), most often TBI plus cyclophosphamide, or a chemotherapy-based regimen (25·1% in BMT, 35·5% in PBSCT), most often busulphan plus cyclophosphamide. The median dose of nucleated cells given in the BMT group was $3·0 \times 10^8$/kg recipient body weight (range $0·3$–$18·4 \times 10^8$/kg). The median number of CD34+ cells infused was $5·0 \times 10^6$/kg recipient body weight ($1·0$–$19·7 \times 10^6$/kg) in the PBSCT group. Prophylaxis for GvHD mainly consisted of a combination of CsA and three doses of short-term MTX (90·2% in BMT, 87·4% in PBSCT). The remaining patients received the four doses (day +1, +3, +6, +11) of MTX (6·8% in BMT, 8·9% in PBSCT) or less than two doses (3·1% in BMT, 3·7% in PBSCT). There were significant differences in the following variables: both patients and donors were older, and chemotherapy-based conditioning regimen was more frequent

Table I. Patient, donor and graft characteristics.

	BM n	BM %	PBSC n	PBSC %	P-value
No. of patients	295		214		
Median patient age, years (range)	38 (16–58)		41 (15–67)		0·028
Patient sex (male/female)	179/116		113/101		0·076
Female donor	137		114		0·137
Female to male	78		58		0·886
Median donor age, years (range)	37 (12–80)		41 (11–71)		0·045
ECOG PS					0·060
0–1	287	97·3	201	93·9	
2–4	8	2·7	13	6·1	
Risk group					0·352
Standard risk	183	62·0	124	57·9	
High risk	112	38·0	90	42·1	
Diagnosis					
Standard risk					0·485
AML	49	26·8	36	29·0	
CML	74	40·4	47	37·9	
ALL	42	23·0	34	27·4	
MDS	18	9·8	7	5·6	
High-risk					0·920
AML	57	50·9	46	51·1	
CML	14	12·5	9	10·0	
ALL	16	14·3	16	17·8	
MDS	15	13·4	10	11·1	
Others	10	8·9	9	10·0	
Conditioning regimen					0·011
TBI-based	221	74·9	138	64·5	
Chemotherapy-based	74	25·1	76	35·5	
Schedule of MTX					0·528
Abbreviated (one or two doses)	9	3·1	8	3·7	
Three doses	266	90·2	187	87·4	
Four doses	20	6·8	19	8·9	
Patient and donor CMV seronegative	23	7·8	6	2·8	0·014

BM, bone marrow; PBSC, peripheral blood stem cell; ECOG PS, Eastern Cooperative Oncology Group performance status; HLA, human leucocyte antigen; AML, acute myeloid leukaemia; ALL, acute lymphoid leukaemia; CML, chronic myeloid leukaemia; TBI, total body irradiation; MDS, myelodysplastic syndrome; GvHD, graft versus host disease; MTX, methotrexate; CMV, cytomegalovirus.

Standard risk disease included AML or ALL in first remission, CML in chronic phase and refractory anaemia. High-risk diseases included all other disease and stages.

in the PBSCT group. However, the two groups did not differ significantly for other patient, disease and transplant-related characteristics. Median follow-up period for the surviving patients at the time of analysis was 15 months in the PBSCT group (3–40 months) and 23 months in the BMT group (1–40 months).

Haematopoietic recovery

Among the patients surviving more than 28 d (BMT, $n = 287$; PBSCT, $n = 208$), engraftment occurred in 286 (99·7%) of the BMT patients and in 206 (99·0%) of the PBSCT patients. Patients who received PBSCT had significantly faster

neutrophil and platelet recovery. The median time to a neutrophil count of at least $0·5 \times 10^9/l$ was 16 d (interquartile range 14–19 d) for the BMT group and 14 d (interquartile range 12–16 d) for the PBSCT group. The median time to a platelet count of at least $20 \times 10^9/l$ was 22 d (interquartile range 18–28 d) for the BMT group and 18 d (interquartile range 13–25 d) for the PBSCT group. In multivariate Cox analyses, PBSCT was significantly associated with faster neutrophil recovery to at least $0·5 \times 10^9/l$ compared with BMT [hazard ratio (HR) = 1·84, 95% CI 1·53–2·22, $P < 0·001$; Table II]. On the contrary, the high-risk disease (HR = 0·73, 95% CI 0·61–0·89, $P = 0·001$) was associated with slower neutrophol recovery. Likewise, the significant factor associated

Outcomes	Analysis	Variables	HR (95% CI)	P-value
Neutrophils >0·5 × 10⁹/l	Cox	Stem cell source: PBSCT	1·84 (1·53–2·22)	<0·001
		Disease risk: high	0·73 (0·61–0·89)	0·001
	IPTW	Stem cell source: PBSCT	1·77 (1·57–2·00)	<0·001
Platelets >20 × 10⁹/l	Cox	Stem cell source: PBSCT	1·52 (1·25–1·84)	<0·001
		Donor age: ≥40 years	0·75 (0·57–0·98)	0·033
	IPTW	Stem cell source: PBSCT	1·46 (1·29–1·66)	<0·001
Grades II–IV acute GvHD	Cox	Stem cell source: PBSCT	1·13 (0·83–1·53)	0·454
	IPTW	Stem cell source: PBSCT	1·14 (0·93–1·41)	0·217
Any grade chronic GvHD	Cox	Stem cell source: PBSCT	1·41 (1·06–1·87)	0·017
		Donor age: ≥40 years	1·56 (1·06–2·29)	0·026
		Disease risk: high	1·40 (1·06–1·87)	0·020
		Prior acute GvHD: grades II–IV	1·66 (1·26–2·20)	<0·001
	IPTW	Stem cell source: PBSCT	1·56 (1·30–1·88)	<0·001
Extensive chronic GvHD	Cox	Stem cell source: PBSCT	1·65 (1·15–2·36)	0·007
		Donor age: ≥40 years	1·65 (1·01–2·70)	0·046
		Disease risk: high	1·45 (1·01–2·07)	0·043
		Prior acute GvHD: grades II–IV	2·36 (1·68–3·33)	<0·001
	IPTW	Stem cell source: PBSCT	1·88 (1·49–2·39)	<0·001

Table II. Multivariate Cox regression analysis and inverse probability-of-treatment weighted (IPTW) method analysis comparing haematopoietic reconstitution and graft *versus* host disease (GvHD) after bone marrow transplantation (BMT) and peripheral blood stem cell transplantation (PBSCT).

The following covariates were included in the Cox models as explanatory variables; patient and donor age (less than or more than 40 years), sex, sex matching, ECOG PS, disease risk, cytomegalovirus (CMV) serology, stem cell source, conditioning regimen, and doses of MTX. The values of stem cell source and significant covariates are shown.

with faster recovery to a platelet count of at least 20 × 10⁹/l was PBSCT (HR = 1·52, 95% CI 1·25–1·84, P < 0·001; Table II). Significant factors for slower platelet recovery were donor age less than 40 years (HR = 0·75, 95% CI 0·57–0·98, P = 0·033) and high-risk disease (HR = 0·77, 95% CI 0·64–0·94, P = 0·008). Using the IPTW method, we confirmed that PBSCT was significantly associated with faster neutophil and platelet recovery (Table II).

Acute GvHD

Table III summarizes clinical characteristics of patients with acute GvHD and the adjusted cumulative incidence of grades II–IV acute GvHD in the two treatment groups is shown in Fig 1. The cumulative incidence of grades II–IV acute GvHD was 37·4% (95% CI 30·9–43·9) in the PBSCT group and 32·0% (95% CI 26·8–37·2) in the BMT group. By multivariate Cox analysis, haematopoietic stem cell source was not a significant factor for the incidence of grades II–IV acute GvHD (BMT vs. PBSCT: HR = 1·13, 95% CI 0·83–1·53, P = 0·454; Table II). We found no significant factor for the incidence of grades II–IV acute GvHD in our model. This result was the same when we used the IPTW method (Table II). The prevalence of organ involvement was different depending on the stem cell source (Table III). Liver and gastrointestinal involvement was more frequent in PBSCT patients than BMT (liver: 14·1% vs. 7·6%, P < 0·019; gut: 27·3% vs. 19·0%, P < 0·014; Table III), whereas skin involvement was similar between the two groups (46·8% vs. 52·6%, P = 0·207).

Table III. Clinical characteristics of patients with acute GvHD.

	BMT (n = 289)	PBSCT (n = 205)	P-value
Acute GvHD			0·213
Grade 0	125 (43·3)	88 (42·9)	
Grade I	70 (24·2)	37 (18·0)	
Grade II	69 (23·9)	44 (21·5)	
Grade III	22 (7·6)	24 (11·7)	
Grade IV	3 (1·0)	12 (5·9)	
Onset after transplantation among patients with grades II–IV acute GvHD			
Median	21	22	
Interquartile range	13·5–28·5	13–31	
Organ involvement			
Skin	152 (52·6)	96 (46·8)	0·207
Liver	22 (7·6)	29 (14·1)	0·019
Gut	52 (17·9)	56 (27·3)	0·014

GvHD, graft *versus* host disease; BMT, bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation.
Values are given as *n* (%).

Chronic GvHD

The adjusted cumulative incidence of any grade chronic GvHD is shown in Fig 2 and the data on the incidence, severity and organ involvement of chronic GvHD are summarized in Table IV. The risk of any grade chronic GvHD in the first year after transplantation was higher in PBSCT than BMT
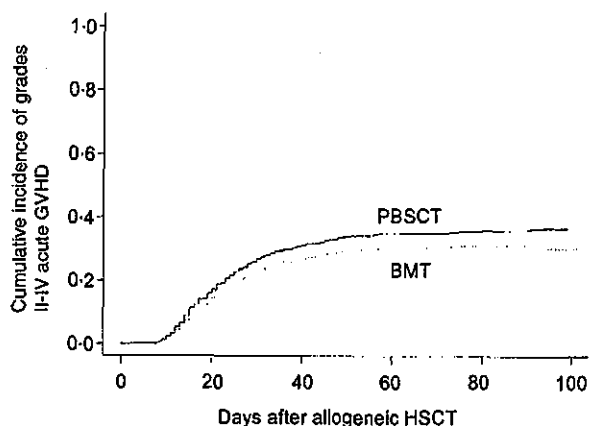
Fig 1. Cumulative incidences of grades II–IV acute graft *versus* host disease (GvHD) after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions were predicted from the proportional subdistribution hazards model and adjusted for effects of significant covariates.

Fig 2. Cumulative incidences of any grade chronic graft *versus* host disease (GvHD) after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions were predicted from the proportional subdistribution hazards model and adjusted for effects of significant covariates, except occurrence of prior grades II–IV acute GvHD.
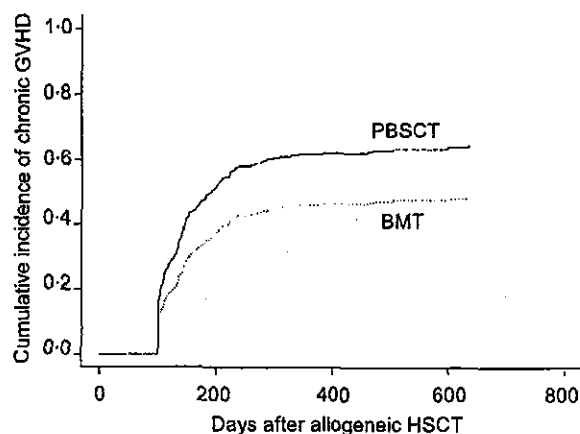
(cumulative incidence at 1 year: 46·2%, 95% CI 40·4–52·4 with BMT vs. 62·1%, 95% CI 54·8–69·4 with PBSCT). The cumulative incidence of limited chronic GvHD was similar in the two groups (19·2%, 95% CI 14·4–24·0 with BMT and 20·2%, 95% CI 14·3–26·0 with PBSCT). However, the extensive form of chronic GvHD was more prevalent in PBSCT than BMT (27·1%, 95% CI 21·5–32·6 with BMT and 41·9%, 95% CI 34·6–49·3 with PBSCT). Progressive and *de novo* forms of chronic GvHD were more frequent in PBSCT. In the multivariate Cox analysis, PBSCT, donor age 40 years or older, high-risk disease and prior grades II–IV acute GvHD were significantly associated with increased risk for any grade

chronic GvHD (BMT vs. PBSCT: HR = 1·41, 95% CI 1·06–1·87, P = 0·017; donor age <40 years vs. ≥40 years: HR = 1·56, 95% CI 1·06–2·29, P = 0·026; standard-risk vs. high-risk disease, HR = 1·40, 95% CI 1·06–1·87, P = 0·02; prior grades 0–I acute GvHD vs. grades II–IV acute GvHD: HR = 1·66, 95% CI 1·26–2·19, P < 0·001; Table II). The extensive form of chronic GvHD was associated with the same risk factors (BMT vs. PBSCT: HR = 1·65, 95% CI 1·15–2·36, P = 0·007; donor age <40 years vs. ≥40 years: HR = 1·65, 95% CI 1·01–2·70, P = 0·046; standard-risk vs. high-risk disease: HR = 1·45, 95% CI 1·01–2·07, P = 0·043; prior grades 0–I acute GvHD vs. grades II–IV acute GvHD: HR = 2·36, 95% CI 1·68–3·33, P < 0·001; Table II). Using the IPTW method, we confirmed a significantly increased incidence of any grade and extensive chronic GvHD in PBSCT group. There were differences in the distribution of organ involvement in chronic GvHD during the course of the disease. Rash/scleroderma (38·9% vs. 25·2%, P = 0·006), oral mucositis (45·0% vs. 22·3%, P < 0·001), ocular sicca (28·9% vs. 15·0%, P = 0·002), and liver abnormality (47·0% vs. 30·6%, P = 0·002) were more frequent in PBSCT patients than in BMT patients. The prevalence of organ involvement was otherwise similar in the two groups (Table IV).

Transplantation-related mortality

The cumulative incidence of TRM at 100 d was 9·7% (95% CI 7·0–12·5) with BMT and 15·0% (95% CI 11·6–18·4) with PBSCT, and at 1 year 16·2% (95% CI 12·3–20·1) with BMT and 19·3% (95% CI 14·1–24·4) respectively (Fig 3; Table V). The stem cell source did not affect TRM in the multivariate Cox, or the IPTW method, analysis. The significant adverse risk factor was grades II–IV acute GvHD (HR = 4·92, 95% CI 2·57–9·42, P < 0·001) at 100 d. At 1 year, donor age 40 years or older (HR = 1·98, 95% CI 1·03–3·80, P = 0·040) and grades II–IV acute GvHD (HR = 2·58, 95% CI 1·65–4·05, P < 0·001) increased the risk of TRM. There were 104 deaths in the BMT group and 75 deaths in the PBSCT group (Table VI). The number of TRM was 51 following BMT (49·0%) and 44 following PBSCT (58·7%), and there was a higher incidence of GvHD-related death in the PBSCT group than in the BMT group (17·3% vs. 3·8%). On the contrary, the number of deaths from relapse was lower in PBSCT (n = 31, 41·3%) than in BMT (n = 53, 51·0%). Time to non-relapse death was similar in the two groups.

Relapse

For the standard-risk group, the cumulative incidence of relapse at 1 year was similar (8·1%, 95% CI 4·2–12·0 with BMT vs. 7·5%, 95% CI 3·1–11·9 with PBSCT; Fig 4A). For the high-risk group, this was 37·1% (95% CI 28·0–46·4) with BMT and 33·3% (95% CI 23·3–43·4) with PBSCT respectively (Fig 4B). In multivariate Cox analysis, there was no statistical difference in the risk of relapse after PBSCT and BMT (HR = 0·95, 95%

Table IV. Clinical characteristics of patients with chronic GvHD.

	BMT (n = 206)	PBSCT (n = 149)	P-value
The incidence of chronic GvHD			0·001
All grade	113 (54·9)	107 (71·8)	
Limited	47 (22·8)	33 (22·1)	
Extensive	66 (32·0)	74 (49·7)	
Onset after transplantation among patients with chronic GvHD (days)			
Median	131	127	
Range	100–634	100–598	
Type			0·003
Progressive	12 (5·8)	15 (10·1)	
Quiescent	59 (28·6)	43 (28·9)	
De novo	42 (20·4)	49 (32·9)	
Organ involvement			
Rash/scleroderma	52 (25·2)	58 (38·9)	0·006
Oral mucositis	46 (22·3)	67 (45·0)	<0·001
Ocular sicca	31 (15·0)	43 (28·9)	0·002
Pulmonary disease	14 (6·8)	19 (12·8)	0·057
Liver abnormalities	63 (30·6)	70 (47·0)	0·002
Nausea/vomiting	6 (2·9)	10 (6·7)	0·089
Diarrhoea	7 (3·4)	7 (4·7)	0·534
Esophagitis	2 (1·0)	3 (2·0)	0·411
Arthralgias/arthritis	5 (2·4)	6 (4·0)	0·112
Effusions	1 (0·5)	1 (0·7)	0·818
Auto-antibody	2 (1·0)	2 (1·3)	0·744
Thrombocytopenia (<100 × 10⁹/l)	38 (19·3)	38 (26·6)	0·112

GvHD, graft *versus* host disease; BMT, bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation.
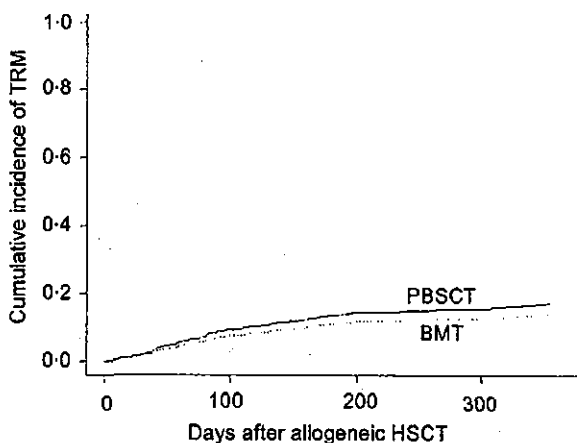Values are given as *n* (%).

Fig 3. Cumulative incidences of treatment-related mortality after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions were predicted from the proportional subdistribution hazards model and adjusted for effects of significant covariates.

CI 0·64–1·41, $P = 0·806$; Table V). We found that the high-risk disease (HR = 3·97, 95% CI 2·66–5·94, $P < 0·001$) and

ECOG PS 2–4 (HR = 3·42, 95% CI 1·73–6·77, $P < 0·001$) had a significantly increased risk of relapse. We did not observe any difference of relapse between the PBSCT and BMT groups using the IPTW method.

Progression-free and overall survival

In standard risk patients, the 2-year PFS and OS in PBSCT and BMT were, respectively, 68·2% (95% CI 58·8–77·5) and 64·7% (95% CI 57·0–72·5) ($P = 0·993$), and 74·1% (95% CI 65·2–83·1) and 73·8% (95% CI 66·9–80·6) ($P = 0·991$). In high-risk patients, PFS and OS in PBSCT and BMT were, respectively, 34·9% (95% CI 23·7–46·0) and 37·7% (95% CI 27·7–47·7) ($P = 0·539$), and 39·1% (95% CI 27·5–50·8) and 44·5% (95% CI 34·3–54·6) ($P = 0·555$; Fig 5A,B). In the multivariate Cox analysis, the use of PBSCT was not a significant factor for both PFS and OS (Table V). We obtained the same result using the IPTW method. The following variables were significant adverse risk factors for both PFS and OS, respectively: high-risk disease (HR = 2·41, 95% CI 1·82–3·21, $P < 0·001$; HR = 2·45, 95% CI 1·79–3·34, $P < 0·001$), ECOG PS 2–4 (HR = 2·83, 95% CI 1·63–4·92, $P < 0·001$; HR = 3·31, 95% CI 1·88–5·84, $P < 0·001$), and grades II–IV acute GvHD (HR = 1·33, 95% CI 1·00–1·78, $P = 0·05$; HR = 1·57, 95% CI 1·15–2·13, $P = 0·004$).

Discussion

This is the first large comparative study from an Asian area on the outcome of allogeneic HSCT using different sources of stem cells (BMT or PBSCT). We analysed the outcome of allogeneic HSCT from related donors in 509 Japanese patients with leukaemia and MDS. All of the patients in our cohort were given G-CSF postgrafting and we confirmed the more rapid haematological recovery after PBSCT than in BMT, which is in line with many previous studies (Schmitz *et al*, 1998, 2002; Champlin *et al*, 2000; Heldal *et al*, 2000; Powles *et al*, 2000; Bensinger *et al*, 2001; Cutler *et al*, 2001; Couban *et al*, 2002; Ringden *et al*, 2002).

It has been suggested that the increased incidence of acute GvHD in PBSCT patients is a consequence of PBSC grafts containing 1 log more T cells compared with bone marrow grafts, although this may be counterbalanced by the decreased potential of type 1 cytokine secretion from donor T cells in PBSC grafts (Mielcarek *et al*, 1997). In clinical studies, a statistically significant increase in acute GvHD after PBSCT has been reported in an RCT (Schmitz *et al*, 2002) and a meta-analysis (Cutler *et al*, 2001). On the contrary, there was no difference in other RCTs (Heldal *et al*, 2000; Powles *et al*, 2000; Bensinger *et al*, 2001; Couban *et al*, 2002). We also found no increased incidence of grades II–IV acute GvHD after PBSCT in the current study. Another important point to be discussed is the dose of MTX that was used as prophylaxis for GvHD. The most common regimen for MTX in Japanese institutions in HLA-identical-related donor transplantation is