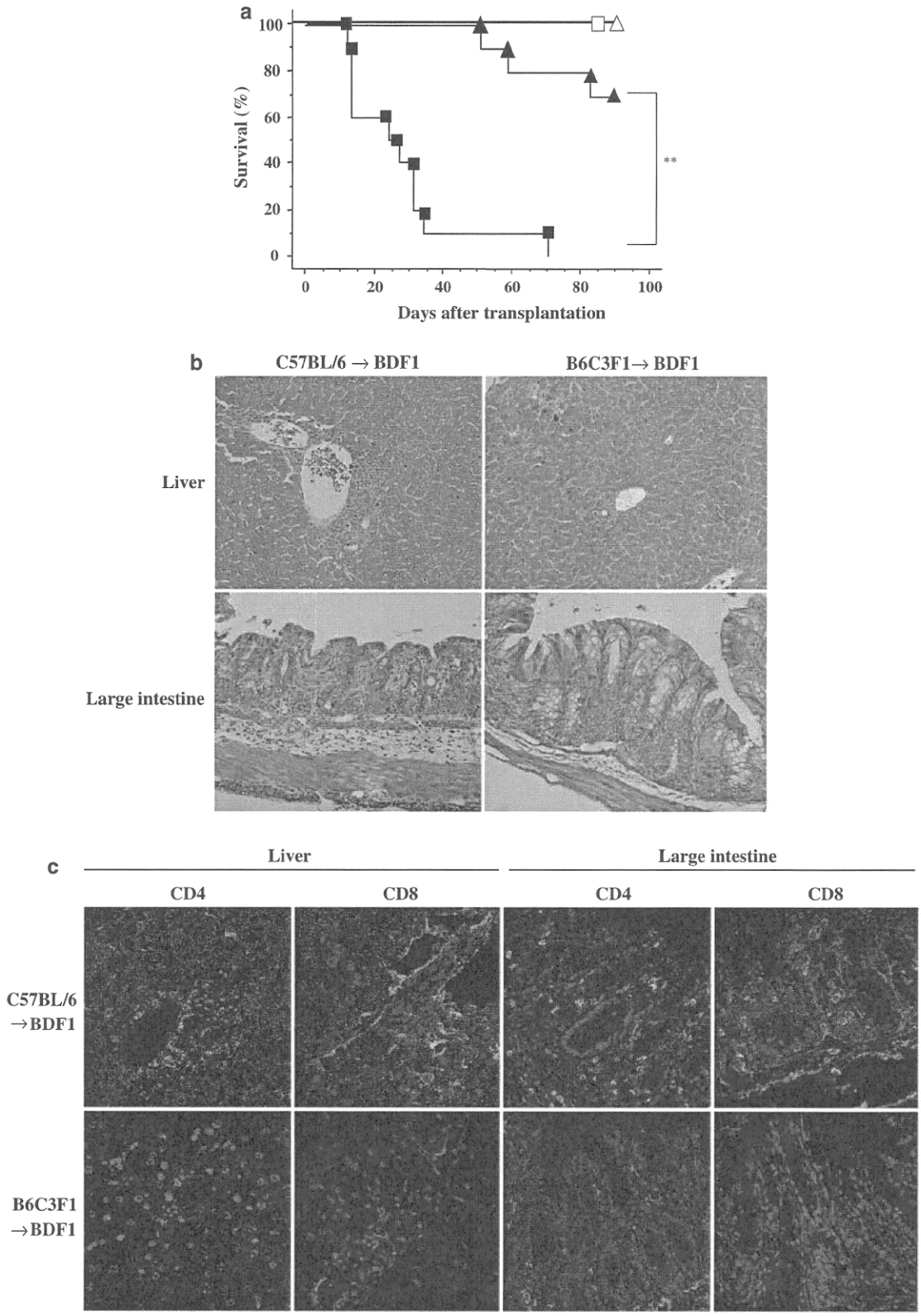


out to other organs, including the humeral bones and mesenteric lymph nodes, until death. Once mice became positive for fluorescence tumor signals, they always succumbed to tumor. We killed some mice, and confirmed mCherry-positive P815 cells in the liver, spleen, and abdominal lymph nodes, as well as the bone marrow in the femoral, sternal,

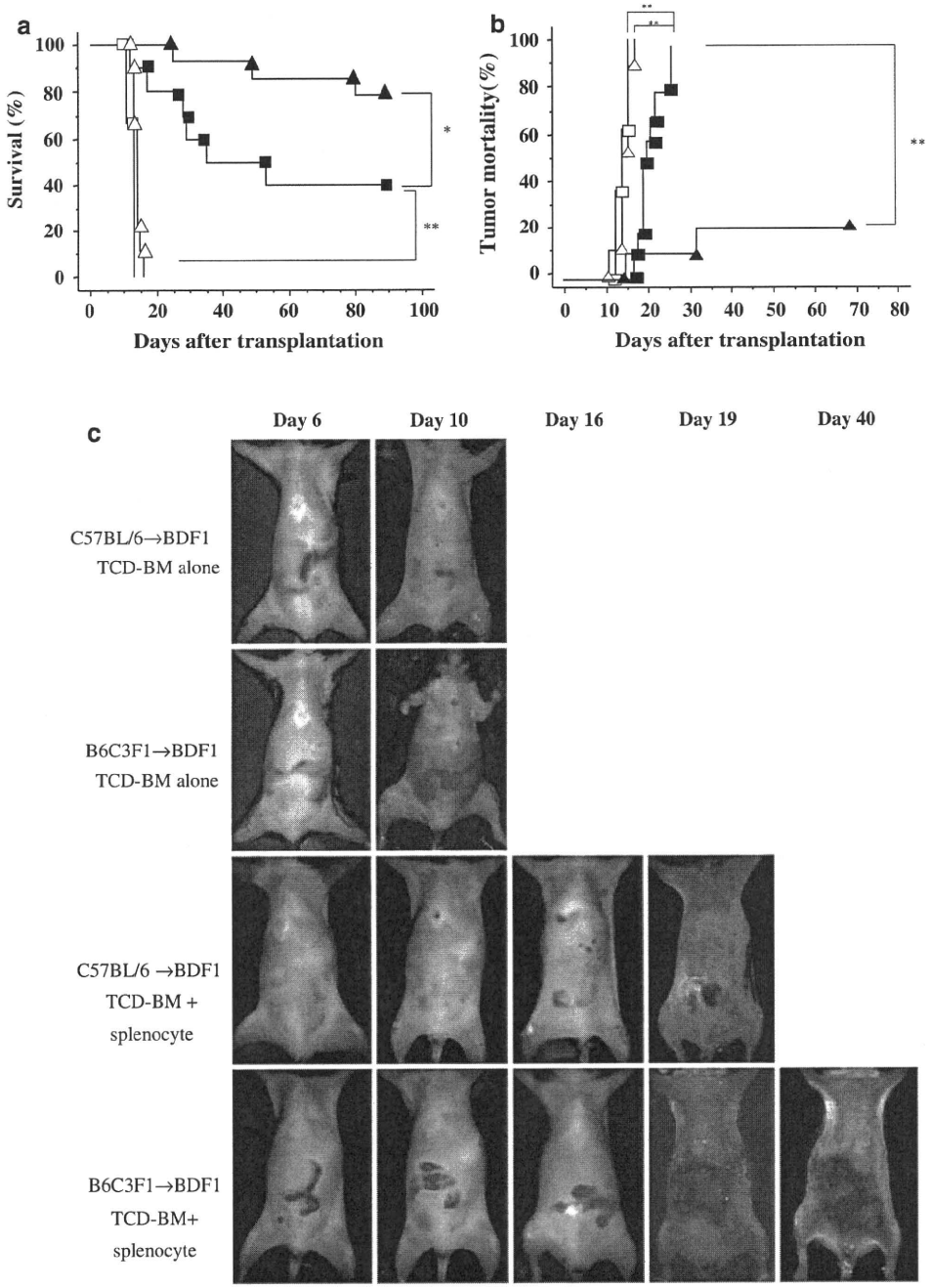


Fig. 2 B6C3F1 → BDF1 recipients developed stronger antileukemic effects than C57BL/6 → BDF1 recipients. **a** BDF1 mice received P815 mastocytoma cells (H-2^d, 1×10^4) with donor TCD-BM cells (5×10^6) with or without donor spleen cells (2×10^7) after receiving TBI 9 Gy the previous day. *Open rectangles* C57BL/6 → BDF1 mice receiving TCD-BM cells and P815 cells ($n = 6$), *open triangles* B6C3F1 → BDF1 receiving TCD-BM cells and P815 cells ($n = 9$), *closed rectangles* C57BL/6 → BDF1 mice receiving TCD-BM, spleen cells and P815 cells ($n = 10$), *closed triangles* B6C3F1 → BDF1 receiving TCD-BM, spleen cells and P815 cells ($n = 14$). *P value < 0.05. Representative data from 3 separate experiments are shown. **b** The same experiments as in **a** were performed except for the reduced number of spleen cells transplanted to 5×10^5. Each *symbol* indicates the same mice as shown in **a**. **P value < 0.01. Representative data from 3 separate experiments are shown. **c** In vivo imaging analysis confirmed the difference in the kinetics of tumor progression between the 2 BMT groups. Transplantation was performed in the same condition as shown in **b**. P815 cells were engineered to express mCherry fluorescent protein by a lentiviral gene transduction system (see "Materials and methods"). Fluorescent imaging in mice was checked every other day from days 6 to 21, and thereafter once a week until day 40. Tumor mass of P815 cells and gastrointestinal contents are visualized as *red* and *blue*, respectively. Representative images from 2 independent experiments are shown

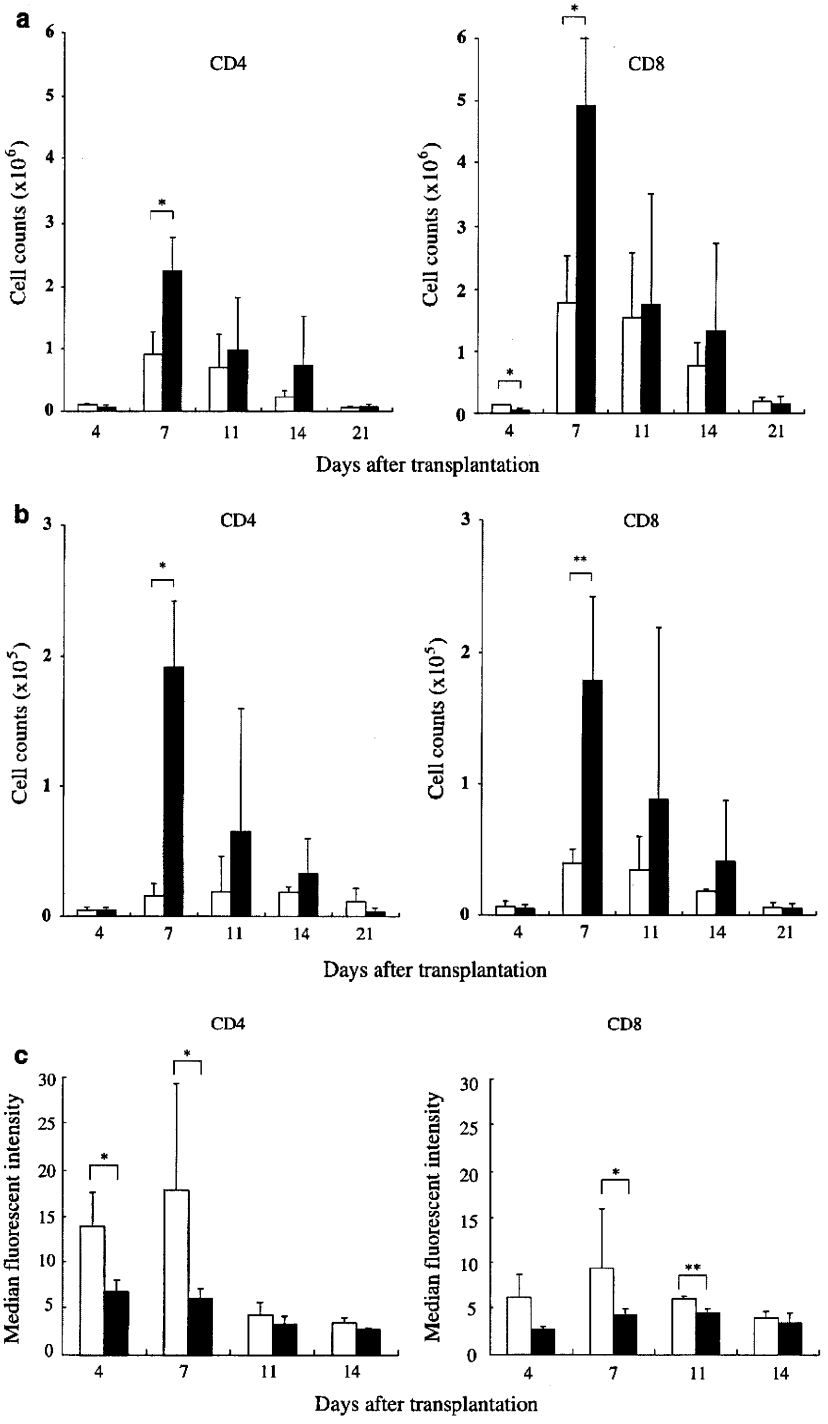


Fig. 3 The kinetics and characterization of T cells proliferating in recipient spleen. **a** Kinetic analysis of donor T cells engrafted to recipient spleens. B6C3F1 → BDF1 and C57BL/6 → BDF1 BMTs were performed as shown in Fig. 1. The number of donor $CD4^+$ or $CD8^+$ T cells was calculated based on multi-colored flow cytometry data, as described in "Materials and methods". *Open bars* C57BL/6 → BDF1 recipients, *closed bars* B6C3F1 → BDF1 recipients. Values were calculated based on experiments using at least 4 mice. Data are expressed as the mean ± standard deviation (SD). $*P < 0.05$. Representative data from 2 separate experiments are shown. **b** Kinetic analysis of host T cells recruited to recipient spleens. Each *symbol* indicates the same mice as shown in **a**. $*P < 0.05$, $**P < 0.01$. Representative data from 2 separate experiments are shown. **c** Median fluorescent intensity of CXCR3 expression on donor $CD4^+$ and $CD8^+$ T cells was compared in the 2 groups ($n = 3$). *Open bars* C57BL/6 → BDF1 recipients, *closed bars* B6C3F1 → BDF1 recipients. Results are representative of 2 experiments. $*P < 0.05$, $**P < 0.01$

and humeral bones by fluorescence microscope and flow cytometry (data not shown). On the other hand, no detectable signals were observed in most B6C3F1 → BDF1 recipients receiving TCD-BM and spleen cells during the observation period. These results demonstrated that B6C3F1 → BDF1 recipients developed more powerful antileukemic effects despite presenting with less severe GVHD compared with C57BL/6 → BDF1 recipients.

3.3 Lower expression of CXCR3 on donor T cells engrafted to B6C3F1 → BDF1 spleens was associated with less severe GVHD

To address the difference in the extent of GVHD between B6C3F1 → BDF1 and C57BL/6 → BDF1 recipients (Fig. 1), we examined the kinetics of and characterized donor T cells engrafted to recipient spleens by flow

cytometry. As shown in Fig. 3a, $CD8^+$ T cells dominated $CD4^+$ T cells in the observation period, and donor T cells, being fewer on day 4, rapidly increased to peak on day 7, and thereafter decreased rapidly or gradually. On day 4, the number of $CD8^+$ T cells in C57BL/6 → BDF1 recipients was significantly greater than in B6C3F1 → BDF1 recipients. On day 7, the number of $CD4^+$ and $CD8^+$ T cells in B6C3F1 → BDF1 recipients was significantly greater than that in C57BL/6 → BDF1 recipients. Regarding the kinetics of host T cells recruited to spleens, although 1 or 2 orders of magnitude lower than donor T cells, the number of host $CD4^+$ and $CD8^+$ T cells rapidly increased to peak on day 7, and thereafter decreased gradually (Fig. 3b). The numbers of $CD4^+$ and $CD8^+$ T cells on day 7 were significantly greater in B6C3F1 → BDF1 recipients than those in C57BL/6 → BDF1 recipients.

We next examined the CXCR3 expression status on donor T cells in recipient spleens. CXCR3 is a Th1-associated chemokine receptor, which plays an important role in the homing of donor T cells to GVHD-target organs [21, 22]. CXCR3 expression levels on donor T cells were highest on day 4, and thereafter decreased rapidly or gradually. Compared with B6C3F1 → BDF1 recipients, C57BL/6 → BDF1 recipients showed a significantly higher median fluorescent intensity of CXCR3 expression in $CD4^+$ T cells on days 4 and 7, and in $CD8^+$ T cells on days 7 and 11 (Fig. 3c). Regarding the expression of CCR5, another Th1-associated chemokine receptor, on donor T cells, the median fluorescent intensity of donor $CD4^+$ or $CD8^+$ T cells was also significantly higher in C57BL/6 → BDF1 recipients than in B6C3F1 → BDF1 recipients in the early transplantation days (data not shown). Thus, a relatively low CXCR3 or CCR5 expression on donor T cells proliferating in recipient spleen was considered to be associated with the occurrence of less severe GVHD in B6C3F1 → BDF1 recipients.

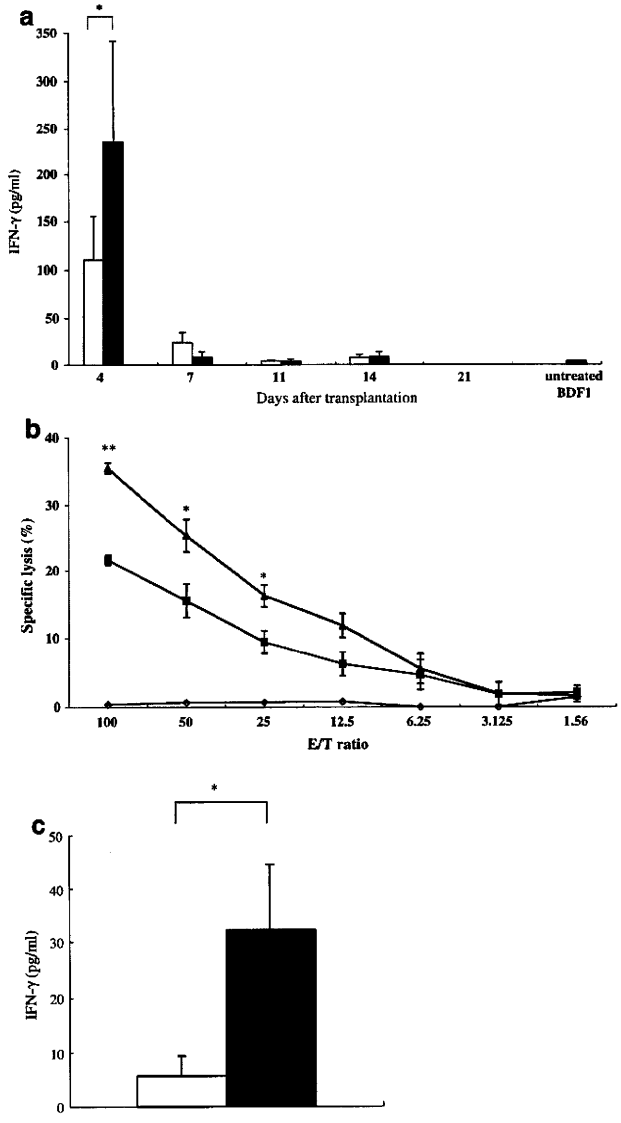
3.4 Stronger in vitro CTL activities and high IFN-γ expression in B6C3F1 → BDF1 recipients

To address the mechanism of stronger antileukemic activity in B6C3F1 → BDF1 recipients (Fig. 2), we next compared the production of inflammatory cytokines, TNF-α and IFN-γ, between B6C3F1 → BDF1 and C57BL/6 → BDF1 recipients. TNF-α is a well-known inflammatory cytokine to play a major role in inducing GVHD [23], while IFN-γ was recently reported to induce the separation of GVL effects from GVHD [11]. Serum TNF-α levels peaked on day 7, and thereafter decreased. There was no significant difference in serum TNF-α levels between the 2 groups (data not shown). On the other hand, serum IFN-γ levels were highest on day 4, and thereafter rapidly decreased (Fig. 4a). Serum IFN-γ levels on day 4 in B6C3F1 → BDF1 recipients were significantly higher than in C57BL/6 → BDF1 recipients.

To ensure powerful antileukemic effects in B6C3F1 → BDF1 recipients, we performed an in vitro cytotoxicity assay against P815 cells. Using, as responders, spleen cells that were recovered from the recipient mice on day 14 in the experiment in Fig. 2a, we compared B6C3F1 → BDF1 and C57BL/6 → BDF1 BMTs. Responder cells used for cytotoxicity assay of C57BL/6 → BDF1 BMT were composed of a mean of 72.6% $CD3^+$ cells (99.7% donor type; 18.0% $CD4^+$ cells and 82.0% $CD8^+$ cells) and a mean of 18.7% NK cells (88.2% donor type). Those of B6C3F1 → BDF1 BMT were composed of a mean of 71.2% $CD3^+$ cells (100% donor type; 22.3% $CD4^+$ cells and 77.7% $CD8^+$ cells) and a mean of 9.7% NK cells (55.4% donor type). There was no significant difference in the percentage of $CD4^+$ or $CD8^+$ cells between C57BL/6 → BDF1 and B6C3F1 → BDF1 spleen cells. Recipient NK cell numbers in B6C3F1 → BDF1 spleen were significantly greater (around twice) than those in C57BL/6 → BDF1 spleen. As shown in Fig. 4b, spleen cells from B6C3F1 → BDF1 recipients showed significantly stronger CTL activities than those from C57BL/6 → BDF1 recipients. In addition, spleen cells recovered from recipients on day 14 were co-cultured with irradiated BDF1 spleen cells for 3 days, and we measured the IFN-γ concentration in the culture supernatant (Fig. 4c). Significantly higher IFN-γ production was observed in culture supernatants from B6C3F1 → BDF1 recipients than in those from C57BL/6 → BDF1 recipients. These results indicate that, compared with C57BL/6 → BDF1 recipients, B6C3F1 → BDF1 recipients have more powerful antileukemic effects, and also suggest that high IFN-γ production may have been involved in the induction of the powerful antileukemic effects.

3.5 Remarkable contribution of recipient immune cells to high IFN-γ production

To address the mechanism of high IFN-γ production in B6C3F1 → BDF1 recipients, we next intended to identify IFN-γ-secreting cells in recipient spleens. IFN-γ-secreting cells were calculated based on flow cytometry data using intracellular IFN-γ staining (Fig. 5a). In the analysis of whole mononuclear cells, on day 4, host-derived IFN-γ-secreting cells were significantly increased in B6C3F1 → BDF1 recipients than in C57BL/6 → BDF1 recipients but not donor-derived cells. On day 7, the number of IFN-γ-secreting cells in B6C3F1 → BDF1 recipients was significantly higher in both donor and host populations compared with in C57BL/6 → BDF1 recipients (Fig. 5b). In the analysis of each donor-derived lineage cell, there was no significant difference in the number



◀ **Fig. 4** Stronger in vitro CTL activity and higher IFN-γ expression in B6C3F1 → BDF1 recipients. **a** Serum IFN-γ concentration in recipient mice. B6C3F1 → BDF1 and C57BL/6 → BDF1 BMTs were performed as shown in Fig. 1. Serum IFN-γ concentration was consecutively measured by Bio-Plex (see "Materials and methods"). *Open bars* C57BL/6 → BDF1 recipients ($n = 8$), *closed bars* B6C3F1 → BDF1 recipients ($n = 8$), *gray bar* untreated BDF1 mice ($n = 3$). Serum IFN-γ levels on day 21 became undetectable in the 2 BMT groups. Data are expressed as the mean ± SD. $*P < 0.05$. Representative data from 2 separate experiments are shown. **b** In vitro cytotoxicity assay for P815 cells was performed using spleen cells on day 14. To obtain responders, mice received TCD-BM (5×10^6) and spleen (2×10^7) cells with P815 cells (1×10^4) after receiving TBI 9 Gy, as shown in Fig. 2a. Spleen cells were recovered from recipient mice on day 14, and directly checked for CTL activity against P815 cells by ^{51}Cr release assay, as described in "Materials and methods". *Closed triangles* B6C3F1 → BDF1 recipients, *closed rectangles* C57BL/6 → BDF1 recipients, *closed diamonds* untreated BDF1 spleen cells. Values were calculated based on experiments using at least 4 samples. Data represent the mean ± SD of specific lysis percentage for P815 cells at a given E/T ratio. Representative results from 2 independent experiments are shown. **c** IFN-γ production in the culture supernatants of mixed lymphocyte culture. B6C3F1 → BDF1 and C57BL/6 → BDF1 BMTs were performed as shown in Fig. 1. Spleen cells were recovered from recipient mice on day 14, and were co-cultured with irradiated (20 Gy) BDF1 spleen cells for 3 days, and the culture supernatant was checked for IFN-γ concentration by Bio-Plex (see "Materials and methods"). *Open bar* C57BL/6 → BDF1 recipients, *closed bar* B6C3F1 → BDF1 recipients. Values were calculated based on experiments using 5 samples. Data are expressed as the mean ± SD. $*P < 0.05$. Representative results from 2 independent experiments are shown

of IFN-γ-secreting cells between the 2 BMT recipients except for $CD8^+$ T cells on day 7 (Fig. 5c). When we calculated based on the donor T cell counts shown in Fig. 3a, the majority of $CD8^+$ T cells in the 2 BMT recipients on day 4 were IFN-γ-secreting cells, with IFN-γ-secreting cells rapidly decreasing to only 10–15% on day 7. In the analysis of each host-derived lineage cell, IFN-γ-secreting cells were significantly increased in $CD8^+$ T cells on day 7 and in NK cells on days 4 and 7 of B6C3F1 → BDF1 recipients compared with C57BL/6 → BDF1 recipients (Fig. 5d). In particular, the number of IFN-γ-secreting host NK cells on day 4 in B6C3F1 → BDF1 recipients was 4 times higher than in C57BL/6 → BDF1 recipients. These data strongly indicate that recipient immune cells, including T and NK cells, have highly contributed to high IFN-γ production in B6C3F1 → BDF1 recipients.

4 Discussion

In the present study, using MHC-haploidentical murine BMT models, we showed that, compared with C57BL/6 → BDF1 (homo-to-hetero-type BMT) recipients, B6C3F1 → BDF1 (hetero-to-hetero-type BMT) recipients showed stronger antileukemic effects with less severe GVHD (Figs. 1, 2). Using these models, we addressed the mechanism by which B6C3F1 → BDF1 BMT exerted stronger antileukemic effects with less severe GVHD than C57BL/6 → BDF1 BMT, because clarification of the mechanism is considered to be useful for the understandings of the separation of GVL effects from GVHD in MHC-haploidentical HSCT.

Significantly higher antileukemic activity of B6C3F1 → BDF1 recipients was also confirmed by in vitro cytotoxicity assay against P815 cells using spleen cells (Fig. 4b). We speculated that high IFN-γ production was involved in this higher antileukemic activity of B6C3F1 → BDF1 recipients. In fact, serum IFN-γ levels in the early transplant period (on day 4) were significantly higher in B6C3F1 → BDF1 recipients than in C57BL/6 → BDF1 recipients (Fig. 4a), and IFN-γ levels in the culture supernatant for MLC were significantly higher than in C57BL/6 → BDF1 recipients (Fig. 4c). We consider that this high IFN-γ production is not the effect but the cause of strong

allogeneic response. IFN-γ has been shown to enhance Th1 polarization through the facilitation of production of IL-12 by dendritic cells (DC) [24] or through synergizing with T cell receptor signals to maximally induce T-bet [25]. Furthermore, we demonstrated that host immune cells, especially NK cells, recruited to the spleen, substantially contributed to high IFN-γ production in B6C3F1 → BDF1 recipients (Fig. 5). The fact that host NK cells still remained in recipient spleens on day 14 and the fact that the number of the host NK cells was significantly greater in B6C3F1 → BDF1 spleens than in C57BL/6 → BDF1 spleens support that host NK cells were more involved in high IFN-γ production in B6C3F1 → BDF1 recipients. In particular, high IFN-γ production in spleen, a secondary lymphoid organ, is considered to be important for inducing strong antileukemic response. Although NK cells are largely excluded from lymph nodes under steady-state conditions, recruitment of NK cells to antigen-stimulated lymph nodes was recently reported to be important for providing an early source of IFN-γ that is necessary for Th1 polarization [26]. NK cells are also shown to participate in adaptive immune responses by modulating DC function or by producing IFN-γ [27].

Furthermore, IFN-γ has been reported to enhance GVL effects mainly by the following 2 mechanisms: the augmentation of lymphohematopoietic GVH reactions [10–12, 28] and enhancement of the susceptibility of tumor cells to alloresponses. Although the mechanism by which IFN-γ enhances lymphohematopoietic GVH reactions remains to be determined, this cytokine may mediate antihematopoietic activity that predominantly targets the recipient hematopoietic cells through its direct effect on hematopoietic cells [29]. IFN-γ may enhance the sensitivity of tumor cells to cytotoxic donor T cells by upregulating surface expressions of Fas and Fas-L, or MHC on tumor cells [30–32], or its direct effect on tumor cells [33].

The analysis of chemokine receptors [21, 22] on donor T cells engrafted to recipient spleens revealed that $CD4^+$ or $CD8^+$ T cells from B6C3F1 → BDF1 recipients showed significantly lower CXCR3 expression levels, compared with those from C57BL/6 → BDF1 recipients (Fig. 3c). These donor T cells in B6C3F1 → BDF1 recipients, which also showed lower CCR5 expression (data not shown), were considered to have low ability to home to GVHD-target organs, which is a prerequisite for the induction of GVHD, despite highly expanding in the spleen. There was no significant difference in the serum levels of TNF-α, a major inducer of GVHD [23], between the 2 groups.

Regarding GVHD-protective effects of IFN-γ, IFN-γ inhibits T cell activation through various mechanisms, including the induction of apoptosis to alloreactive T cells [34], the inhibition of Th17 cells [35], the generation of CD4 regulatory T cells [36], and the activation of immunosuppressive mesenchymal stem cells [37, 38], and furthermore the direct interaction of IFN-γ with recipient pulmonary parenchyma was recently reported to prevent idiopathic pneumonia syndrome in lethally irradiated mice after allogeneic HSCT [14].

In the experiment using another MHC-haploidentical BMT models, C3D2F1 ($H\text{-}2^{k/d}$) → B6C3F1 ($H\text{-}2^{b/k}$) and C3H ($H\text{-}2^{k}$) → B6C3F1, stronger antileukemic effects for EL4 cells ($H\text{-}2^{b}$) with less severe GVHD were observed in C3D2F1 → B6C3F1 recipients (hetero-to-hetero-type BMT) (data not shown). These findings suggest that separation of GVL effects from GVHD could be a generalized phenomenon observed mainly in hetero-to-hetero-type mismatched BMT; however, we need further experiments to confirm the hypothesis because the severity of GVHD is highly dependent on the strain combination in murine BMT models. Regarding the relationship between the findings in this animal study and our clinical data, we had 4 patients who underwent unmanipulated HLA-2-3 antigen-mismatched HSCT undergone in homo-to-hetero combination: 3 patients received myeloablative conditioning and the remaining 1 reduced-intensity conditioning. Two patients had no acute GVHD, 1 grade II GVHD, and the remaining early death. Among them, only 1 patient had a relapse. Homo-to-hetero combination does not seem to clinically develop severe GVHD, despite the experience of only 4 cases; however, the clinical outcome must have been influenced by intensive GVHD prophylaxis containing steroids in our HLA-haploidentical HSCT regimen [7, 8]. To obtain some clinical evidence, a large scale-study is needed, and we consider that the use of less intensified GVHD prophylaxis in our protocol may make the difference between homo-to-hetero and hetero-to-hetero combinations more evident in unmanipulated HLA-haploidentical transplant settings.

Our findings suggest that two-way in vivo MLC reaction occurs in spleens more strongly in hetero-to-hetero BMT recipients compared with homo-to-hetero BMT recipients, and that host immune cells, such as NK or T cells, produce IFN-γ abundantly, which may be considered to have contributed to the separation of GVL from GVHD. Thus, the participation of host hematopoietic cells in the afferent phase of GVHD changes the balance between allogeneic GVH and GVL responses of donor T lymphocytes, and that appropriate utilization of host immune cells may enable greater separation of GVL from GVH reactions.

In conclusion, we showed, in MHC-haploidentical murine BMT models, that powerful antileukemic effects with less severe GVHD were associated with a high production of IFN-γ, and also data suggesting that host immune cells, including NK cells, played an important role in this IFN-γ production.

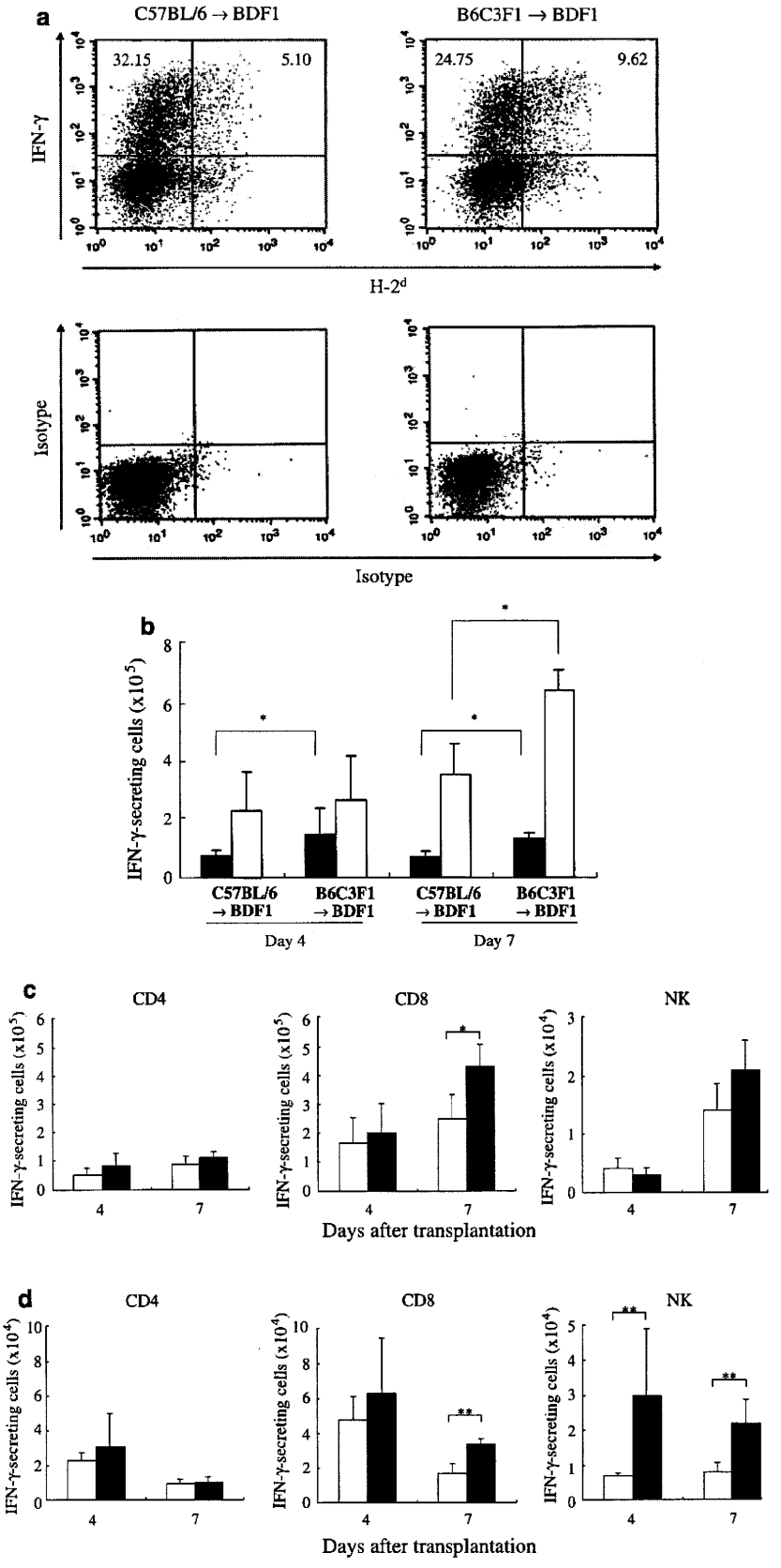


Fig. 5 Contribution of recipient immune cells to high IFN-γ production in B6C3F1 → BDF1 recipients. Transplantation was performed as shown in Fig. 1. IFN-γ-secreting cells in recipient spleens were calculated based on flow cytometry data using intracellular IFN-γ staining. $*P < 0.05$, $**P < 0.01$. **a** Representative flow cytometry data using whole mononuclear cells recovered from spleens on day 4. The *upper panels* indicate the percentages of IFN-γ-secreting cells in the fraction of whole mononuclear cells. *Dot plots* in which isotype-matched control mAb was used are shown in the *lower panels*. A higher percentage of host-derived (H-2^d positive) IFN-γ-secreting cells was observed in B6C3F1 → BDF1 recipients than in C57BL/6 → BDF1 recipients. **b** IFN-γ-secreting cells in the fraction of whole mononuclear cells retrieved from spleens on days 4 ($n = 7$) and 7 ($n = 4$). *Closed bars* host cells, *open bars* donor cells. **c** Donor-derived IFN-γ-secreting cells in each cell lineage. *Open bars* C57BL/6 → BDF1 recipients, *closed bars* B6C3F1 → BDF1 recipients. **d** Host-derived IFN-γ-secreting cells in each cell lineage. *Open bars* C57BL/6 → BDF1 recipients, *closed bars* B6C3F1 → BDF1 recipients. Regarding these IFN-γ-secreting cells, representative results from 2 independent experiments are shown

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Conflict of interest statement The authors have no financial conflicts of interest.

References

1. Thomas ED. Karnofsky memorial lecture. Marrow transplantation for malignant diseases. J Clin Oncol. 1983;1:517–31.
2. Bortin MM, Rimm AA. Increasing utilization of bone marrow transplantation. Transplantation. 1986;42:229–34.
3. Gale RP. Graft-versus-host disease. Immunol Rev. 1985;88:193–214.
4. Ringden O, Nilsson B. Death by graft-versus-host disease associated with HLA mismatch, high recipient age, low marrow cell dose, and splenectomy. Transplantation. 1985;40:39–44.
5. Ikegame K, Tanji Y, Kitai N, Tamaki H, Kawakami M, Fujioka T, et al. Successful treatment of refractory T-cell acute lymphoblastic leukemia by unmanipulated stem cell transplantation from an HLA 3-loci mismatched (haploidentical) sibling. Bone Marrow Transplant. 2003;31:507–10.
6. Ogawa H, Ikegame K, Kawakami M, Tsuboi A, Kim EH, Hosen N, et al. Powerful graft-versus-leukemia effects exerted by HLA-haploidentical grafts engrafted with a reduced-intensity regimen for relapse following myeloablative HLA-matched transplantation. Transplantation. 2004;78:488–9.
7. Ogawa H, Ikegame K, Yoshihara S, Kawakami M, Fujioka T, Masuda T, et al. Unmanipulated HLA 2–3 antigen-mismatched (haploidentical) stem cell transplantation using nonmyeloablative conditioning. Biol Blood Marrow Transplant. 2006;12:1073–84.
8. Ogawa H, Ikegame K, Kaida K, Yoshihara S, Fujioka T, Taniguchi Y, et al. Unmanipulated HLA 2–3 antigen-mismatched (haploidentical) bone marrow transplantation using only pharmacological GVHD prophylaxis. Exp Hematol. 2008;36:1–8.
9. Murai M, Yoneyama H, Ezaki T, Suematsu M, Terashima Y, Harada A, et al. Peyer's patch is the essential site in initiating murine acute and lethal graft-versus-host reaction. Nat Immunol. 2003;4:154–60.
10. Welniak LA, Blazar BR, Anver MR, Wiltrout RH, Murphy WJ. Opposing roles of interferon-gamma on CD4+ T cell-mediated graft-versus-host disease: effects of conditioning. Biol Blood Marrow Transplant. 2000;6:604–12.
11. Yang YG, Qi J, Wang MG, Sykes M. Donor-derived interferon gamma separates graft-versus-leukemia effects and graft-versus-host disease induced by donor CD8 T cells. Blood. 2002;99:4207–15.
12. Wang H, Asavaroengchai W, Yeap BY, Wang MG, Wang S, Sykes M, et al. Paradoxical effects of IFN-gamma in graft-versus-host disease reflect promotion of lymphohematopoietic graft-versus-host reactions and inhibition of epithelial tissue injury. Blood. 2009;113:3612–9.
13. Brok HP, Heidt PJ, van der Meide PH, Zurcher C, Vossen JM. Interferon-gamma prevents graft-versus-host disease after allogeneic bone marrow transplantation in mice. J Immunol. 1993;151:6451–9.
14. Burman AC, Banovic T, Kuns RD, Clouston AD, Stanley AC, Morris ES, et al. IFNγ differentially controls the development of idiopathic pneumonia syndrome and GVHD of the gastrointestinal tract. Blood. 2007;110:1064–72.
15. Cooke KR, Kobzik L, Martin TR, Brewer J, Delmonte J Jr, Crawford JM, et al. An experimental model of idiopathic pneumonia syndrome after bone marrow transplantation. I. The roles of minor H antigens and endotoxin. Blood. 1996;88:3230–9.
16. Yoshida Y, Hirano T, Son G, Iimuro Y, Imado T, Iwasaki T, et al. Allogeneic bone marrow transplantation for hepatocellular carcinoma: hepatocyte growth factor suppresses graft-vs.-host disease. Am J Physiol Gastrointest Liver Physiol. 2007;293:G1114–23.
17. Kubo S, Seleme MC, Soifer HS, Perez JL, Moran JV, Kazazian HH Jr, et al. L1 retrotransposition in nondividing and primary human somatic cells. Proc Natl Acad Sci USA. 2006;103:8036–41.
18. Nakagomi N, Nakagomi T, Kubo S, Nakano-Doi A, Saino O, Takata M, et al. Endothelial cells support survival, proliferation and neuronal differentiation of transplanted adult ischemia-induced neural stem/progenitor cells after cerebral infarction. Stem Cells. 2009;27:2185–95.
19. Barrett T, Koyama Y, Hama Y, Ravizzini G, Shin IS, Jang BS, et al. In vivo diagnosis of epidermal growth factor receptor expression using molecular imaging with a cocktail of optically labeled monoclonal antibodies. Clin Cancer Res. 2007;13:6639–48.
20. Iwasaki T, Fujiwara H, Shearer GM. Loss of proliferative capacity and T cell immune development potential by bone marrow from mice undergoing a graft-vs-host reaction. J Immunol. 1986;137:3100–8.
21. Jaksch M, Remberger M, Mattsson J. Increased gene expression of chemokine receptors is correlated with acute graft-versus-host disease after allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2005;11:280–7.
22. Sackstein R. A revision of Billingham's tenets: the central role of lymphocyte migration in acute graft-versus-host disease. Biol Blood Marrow Transplant. 2006;12:2–8.
23. Hill GR, Ferrara JL. The primacy of the gastrointestinal tract as a target organ of acute graft-versus-host disease: rationale for the use of cytokine shields in allogeneic bone marrow transplantation. Blood. 2000;95:2754–9.
24. Snijders A, Kalinski P, Hilkens CM, Kapsenberg ML. High-level IL-12 production by human dendritic cells requires two signals. Int Immunol. 1998;10:1593–8.
25. Afkarian M, Sedy JR, Yang J, Jacobson NG, Cereb N, Yang SY, et al. T-bet is a STAT1-induced regulator of IL-12R expression in naïve CD4+ T cells. Nat Immunol. 2002;3:549–57.
26. Martín-Fontecha A, Thomsen LL, Brett S, Gerard C, Lipp M, Lanzavecchia A, et al. Induced recruitment of NK cells to lymph nodes provides IFN-γ for TH1 priming. Nat Immunol. 2004;5:1260–5.
27. Moretta A. Natural killer cells and dendritic cells: rendezvous in abused tissues. Nat Rev Immunol. 2002;2:957–64.
28. Pelot MR, Pearson DA, Swenson K, Zhao G, Sachs J, Yang YG, et al. Lymphohematopoietic graft-vs.-host reactions can be induced without graft-vs.-host disease in murine mixed chimeras established with a cyclophosphamide-based nonmyeloablative conditioning regimen. Biol Blood Marrow Transplant. 1999;5:133–43.
29. Rottman M, Soudais C, Vogt G, Renia L, Emile JF, Decaluwe H, et al. IFN-gamma mediates the rejection of haematopoietic stem cells in IFN-gammaR1-deficient hosts. PLoS Med. 2008;5:e26.
30. Böhm W, Thoma S, Leithäuser F, Möller P, Schirmbeck R, Reimann J. T cell-mediated, IFN-gamma-facilitated rejection of murine B16 melanomas. J Immunol. 1998;161:897–908.
31. Sayers TJ, Brooks AD, Lee JK, Fenton RG, Komschlies KL, Wigginton JM, et al. Molecular mechanisms of immune-mediated lysis of murine renal cancer: differential contributions of perforin-dependent versus Fas-mediated pathways in lysis by NK and T cells. J Immunol. 1998;161:3957–65.
32. Puliaev R, Nguyen P, Finkelman FD, Via CS. Differential requirement for IFN-gamma in CTL maturation in acute murine graft-versus-host disease. J Immunol. 2004;173:910–9.

33. Sayers TJ, Wiltrout TA, McCormick K, Husted C, Wiltrout RH. Antitumor effects of alpha-interferon and gamma-interferon on a murine renal cancer (Renca) in vitro and in vivo. Cancer Res. 1990;50:5414–20.
34. Badovinac VP, Tvinnereim AR, Harty JT. Regulation of antigen-specific CD8+ T cell homeostasis by perforin and interferon-gamma. Science. 2000;17:1354–8.
35. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol. 2005;6:1123–32.
36. Sawitzki B, Kingsley CI, Oliveira V, Karim M, Herber M, Wood KJ. IFN-gamma production by alloantigen-reactive regulatory T cells is important for their regulatory function in vivo. J Exp Med. 2005;201:1925–35.
37. Polchert D, Sobinsky J, Douglas GW, Kidd M, Moadsiri A, Reina E, et al. IFN-γ activation of mesenchymal stem cells for treatment and prevention of graft versus host disease. Eur J Immunol. 2008;38:1745–55.
38. English K, Barry FP, Field-Corbett CP, Mahon BP. IFN-gamma and TNF-alpha differentially regulate immunomodulation by murine mesenchymal stem cells. Immunol Lett. 2007;110:91–100.

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ORIGINAL ARTICLE

Use of foscarnet for cytomegalovirus infection after allogeneic hematopoietic stem cell transplantation from a related donor

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Abstract Foscarnet is an active agent against cytomegalovirus (CMV) infection after hematopoietic stem cell transplantation (HSCT), as well as ganciclovir. We investigated the usefulness of foscarnet in patients who underwent related allogeneic HSCT. Foscarnet was used in 320 patients with a median age of 45 years (range 15–72). The purpose of administration was CMV disease in 65, preemptive use in 248 and prophylaxis in 7. Totally, 194 patients had a history of prior ganciclovir treatment. The reason for foscarnet use was insufficient therapeutic effect of prior ganciclovir in 99, and adverse event including myelosuppression in 95. The response rate in symptom was 52% for the CMV disease patients. Antigenemia disappeared in 77% of the preemptive treatment and improved in 13% of the patients. No outbreak of CMV disease was recognized. The total effectiveness of therapeutic and preemptive use was significantly higher for patients without prior ganciclovir (91 vs. 76%, $P = 0.001$). Adverse events of grade 3 or higher were recognized in 24%, including electrolyte abnormalities in 11%, neutropenia in 8%, and thrombocytopenia in 8%. Renal damage was only observed in 3% of patients. Foscarnet was concluded to be a safe and effective anti-CMV agent and to be a suitable alternative to ganciclovir.

Keywords Cytomegalovirus infection · Foscarnet · Blood and marrow transplantation · Efficacy · Adverse reaction

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1 Introduction

Cytomegalovirus (CMV) disease is one of the most important infectious complications after allogeneic hematopoietic stem cell transplantation (HSCT), which influences the outcome of the transplantation. The presence of graft-versus-host disease and steroid therapy are associated with the occurrence of CMV infection or reactivation. Ganciclovir is used as a first-line agent for both prophylaxis and the treatment of CMV disease [1–5]. However, approximately one-third of patients receiving ganciclovir develop drug-induced neutropenia or thrombocytopenia [6–9]. Therefore, ganciclovir is unsuitable for use in patients with poor bone marrow function. Another problem is ganciclovir resistant CMV [10–12].

For such cases, foscarnet is an important alternative agent that demonstrates anti-viral activity against all known herpes viruses including CMV [11, 13–15]. In early studies, the dose-limiting toxicities of foscarnet were found to be nephrotoxicity and neurotoxicity, which were seen in up to 50% of patients [16, 17]. Two randomized controlled trials (RCT) comparing the usefulness of preemptive foscarnet versus ganciclovir have been performed for CMV antigenemia [18, 19]. These studies revealed that the effectiveness of foscarnet was equivalent to that of ganciclovir. Adverse reactions and treatment-related mortality of foscarnet were also the same as those of ganciclovir. Renal dysfunction was only noted in 5% of the patients that received foscarnet [19].

The use of foscarnet has also been reported in cord blood transplantation, which is more complicated by viral infection [20]. These studies including the RCT only involved patients who had received foscarnet as an initial therapy. Therefore, we conducted a nationwide study in Japan of the use of foscarnet against CMV infection after related HSCT to investigate the current status, and compared its efficacy and toxicity in patients with and without prior ganciclovir use.

2 Patients and methods

2.1 Study design

This study is a retrospective survey investigating the use of foscarnet after stem cell transplantation. The subjects of this study were patients who received foscarnet after receiving allogeneic transplantation from a related donor in the period from 1998 to 2008. We performed a questionnaire at institutions carrying out allogeneic stem cell transplants in Japan. Data regarding the presence of CMV disease, CMV antigenemia, the reason for foscarnet use, the dose and duration of foscarnet, the effectiveness of therapy, and adverse events were collected. The obtained data were combined with data from the national registry of the Japan Society of Hematopoietic Cell Transplantation, which was collected by the TRUMP system [21]. This study was approved by the Ethical Committees of the Japan Society of Hematopoietic Cell Transplantation and Hyogo College of Medicine.

2.2 CMV antigenemia assay

Cytomegalovirus antigenemia was measured as described previously [22, 23]. Briefly, peripheral white blood cells were attached to slides by cytocentrifugation and stained with HRP-C7 (Teijin, Tokyo, Japan) or C10/C11 (Biotest, Dreieich, Germany) monoclonal antibodies. The number of positive cells was counted per 50,000 attached cells for HRP-C7 and per 150,000 applied cells for C10/C11. The examination was performed in duplicate, and the mean was used for further analyses.

2.3 Definition of CMV disease and infection

CMV diseases were defined as any organ infections by CMV, ideally proven by histopathologic examinations. They include gastroenteritis, pneumonia, retinitis, hepatitis, encephalitis, and cystitis. Patients who presented with interstitial pneumonia accompanied by CMV antigenemia were also diagnosed with CMV disease (pneumonia). For patients who presented with antigenemia and simultaneous diarrhea, gastrointestinal endoscopy and biopsy were recommended, but those who could not receive such diagnostic procedure were regarded as suspicious CMV disease (gastroenteritis). Both CMV antigenemia and CMV disease were regarded as CMV infection.

2.4 Type of therapy

The administration of anti-viral agents for patients without any CMV disease but accompanied by CMV antigenemia with or without febrile complications was defined as preemptive therapy in this study. Therapy of CMV disease was defined as CMV treatment. The use of anti-viral agents for those without antigenemia or CMV disease was regarded as prophylaxis.

2.5 Statistics

Pairwise comparisons were performed using the χ^2 test and Fisher's exact test for categorical variables, and the Mann–Whitney U test for continuous variables. The Kruskal–Wallis test was used to compare multiple groups. P values of <0.05 obtained in 2-sided tests were considered statistically significant. Data were analyzed with the STATA version 11 statistical software (STATA Corp, TX, USA).

3 Results

3.1 Patient characteristics

The background data of 320 patients are shown in Table 1. There were 171 males and 149 females. Their median age was 45 years, and the ages of the patients ranged from 15 to 72 years. The underlying disease of patients was acute myeloid leukemia (AML) in 110, acute lymphoblastic leukemia (ALL) in 59, chronic myelogenous leukemia (CML) in 18, myelodysplastic syndrome (MDS)/myeloproliferative disorder (MPD) in 42, chronic lymphocytic leukemia (CLL) in 2, non-Hodgkin lymphoma (NHL) in 51, Hodgkin lymphoma (HL) in 4, adult T cell lymphoma (ATL) in 16, multiple myeloma (MM) in 10, aplastic anemia (AA) in 6 and 1 each for renal cell carcinoma and virus associated hemophagocytic syndrome. Several demographic data were not available due to the lack of patient entry to the TRUMP system. CMV antibody was positive in both the patient and donor in 189 pairs (59%), in the patient only in 22 cases (7%), and in the donor only in 8 cases (3%), and it was negative in both patient and donor in 4 pairs (1%). Of 289 patients with evaluable data, 113 patients received bone marrow (BM) as a graft, 172 received peripheral blood stem cell (PBSC), and 4 received both BM and PBSC. HLA was matched in 108 of 268 patients but was mismatched in the remaining 160 (155 with serological mismatch and 5 with allele mismatch).

Table 1 Patient characteristics

Variables	Number
Patient number	320
Median age (range)	45 (15–72)
Male/female	171/149
Disease	
Acute myeloid leukemia	110
Acute lymphoblastic leukemia	59
Chronic myelogenous leukemia	18
Myelodysplastic/myeloproliferative syndrome	42
Chronic lymphocytic leukemia	2
Non-Hodgkin lymphoma	51
Hodgkin lymphoma	4
Adult T cell leukemia	16
Multiple myeloma	10
Aplastic anemia	6
Other diseases	2
CMV serology	
Donor +/Patient +	189
Donor +/Patient −	8
Donor −/Patient +	22
Donor −/Patient −	4
Graft source	
Bone marrow (BM)	113
Peripheral blood stem cell (PBSC)	172
Both BM and PBSC	4
Donor type	
Matched related	108
Mismatched related	160

3.2 CMV infection

Foscarnet was administered for CMV disease in 65 patients (20%), including 46 with gastroenteritis, 12 with pneumonia, 2 with retinitis, and one each for hepatitis, encephalitis, and cystitis. Each one other patient developed pneumonia and retinitis accompanied by simultaneous gastroenteritis. On the other hand, 248 (78%) were preemptively treated (only complicated with CMV antigenemia), and 7 (2%) were prophylactically treated. Before foscarnet administration, 194 (61%) patients had received ganciclovir, and one of the patients was treated with cidofovir after ganciclovir use. The reason for changing the anti-viral agent to foscarnet was insufficient therapeutic effect in 99 patients and adverse events due to preceding ganciclovir including myelosuppression in 95 patients. In 126 patients who had not received any anti-viral premedication, foscarnet was used because of poor bone marrow function in 116.

A total of 208 patients (67%) received steroid therapy at the time of foscarnet initiation. The rate of patients under steroid use was 58% for CMV disease, 70% for preemptive foscarnet, and 43% for prophylaxis, but the difference was not significant ($P = 0.08$).

3.3 Dosage of foscarnet

The initial dose of foscarnet ranged from 7 mg/kg to 216 mg/kg (median 88 mg/kg, Fig. 1). The dose was

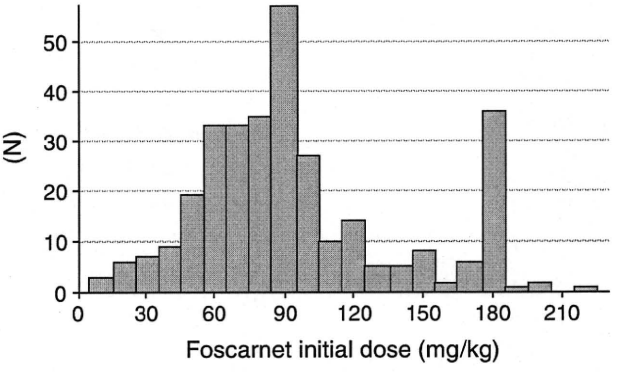

Fig. 1 Initial dose of foscarnet. Foscarnet was given at a variety of doses ranging from 7 to 216 mg/kg (median 88 mg/kg). Two peaks at 90 and 180 mg/kg were seen in the histogram

significantly higher in the patients who had received prior ganciclovir (range 10–216 mg/kg, median 91 mg/kg) than those who had not (range 7–180 mg/kg, median 72 mg/kg) ($P < 0.0001$). The median dose in the preemptive, treatment, and prophylactic groups was 89, 90, and 63 mg/kg, respectively; i.e., it was significantly lower in the prophylactic use group ($P = 0.05$). The initial dose of foscarnet did not have any correlation with creatinine clearance calculated from serum creatinine level and age by the Modification of Diet in Renal Disorder (MDRD) formula ($r = 0.21$, Fig. 2). The duration of foscarnet use ranged from 1 to 163 days (median 20 days) and was significantly shorter for patients who had received prior ganciclovir than those who had not (median 17 vs. 22 days, $P = 0.05$). As there were two peaks at 90 and 180 mg/kg in the dose of foscarnet administered, 5 dose categories (0–39, 40–79, 80–99, 100–159, and 160–220) were defined, and the efficacy and toxicity of foscarnet were estimated according to this categorization.

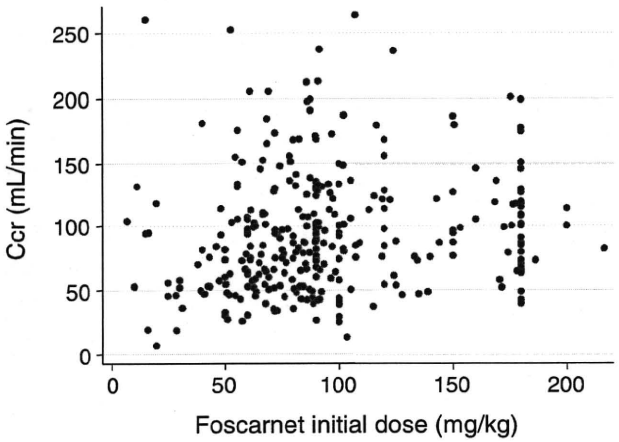

Fig. 2 Relationship between the initial dose of foscarnet and creatinine clearance. Creatinine clearance was calculated from serum creatinine level and age by the Modification of Diet in Renal Disorder (MDRD) formula [Ccr for male = $0.741 \times 175 \times (\text{age})^{(-0.203)} \times (\text{serum creatinine})^{(-1.154)}$, Ccr for female = $0.741 \times 175 \times (\text{age})^{(-0.203)} \times (\text{serum creatinine})^{(-1.154)} \times 0.742$]. No correlation was found ($r = 0.21$)

3.4 Efficacy

Among 65 patients with CMV disease, the symptoms disappeared in 5 (8%) and improved in 28 (44%), no change was seen in 20 (32%), and the symptoms worsened in 10 (16%) (Table 2). One patient was not evaluable with regards to their response, and another patient did not have any symptoms at the initiation of foscarnet because of the effect of prior ganciclovir use. The effectiveness (resolved or improved) was higher in those who did not receive ganciclovir, but the difference was not statistically significant (71 vs. 46%, $P = 0.10$). When the effectiveness in symptom was compared between HLA-matched and -mismatched transplant, the rate was almost comparable (14/25 = 56% vs. 14/29 = 48%, $P = 0.60$). Among 238 evaluable patients who received preemptive CMV therapy, antigenemia was resolved in 183 (77%) and improved in 31 (13%), but was not changed in 17 (7%) and worsened in 7 (3%). No patient developed outbreaks of CMV disease. The effectiveness was higher for those who had not received prior ganciclovir, but the difference was not significant (93/99 = 93% vs. 121/139 = 87%, $P = 0.13$).

Table 2 Response to foscarnet

	Symptoms				Antigenemia			
	Prior GCV		No prior GCV		Prior GCV		No prior GCV	
	N	%	*N*	%	*N*	%	*N*	%
CMV disease								
Disappeared	4	9	1	6	26	65	8	89
Improved/decreased	17	37	11	65	7	18	1	11
No change	18	39	2	12	4	10	0	0
Worsened/increased	7	15	3	18	3	8	0	0
No symptoms/antigenemia	1[a]	–	–	–	7	–	8	–
Unevaluable	1	–	0	–	1	–	0	–
Preemptive								
Disappeared	–	–	105	74	78	80		
Decreased	–	–	17	12	14	14		
No change	–	–	14	10	3	3		
Increased	–	–	5	4	2	2		
No antigenemia	–	–	4[a]	–	–	–		
Unevaluable	–	–	3	–	3	–		

GCV ganciclovir

[a] Symptoms/antigenemia had disappeared after prior GCV

Although the effectiveness in preemptive use was lower in HLA-matched transplant as compared with HLA-mismatched transplant, the difference was not also significant (64/75 = 85% vs. 114/123 = 93%, $P = 0.14$). Among the patients who received prior ganciclovir, the effectiveness was significantly higher in the patients in whom an insufficient effect of ganciclovir was seen compared with those who had suffered an adverse reaction to ganciclovir (64/68 = 94% vs. 57/71 = 80%, $P = 0.02$). The overall effectiveness of treatment and preemptive use was significantly higher in those who had not received prior ganciclovir (91 vs. 76%, $P = 0.001$) because of the low effectiveness in the patients of the CMV disease group who had received prior ganciclovir use. The changing courses of CMV antigenemia are box plotted in Fig. 3a for the patients who received prior ganciclovir and in Fig. 3b for those who did not. After the administration of foscarnet, the CMV antigenemia decreased in both groups ($P < 0.0001$ and $P = 0.01$, respectively).

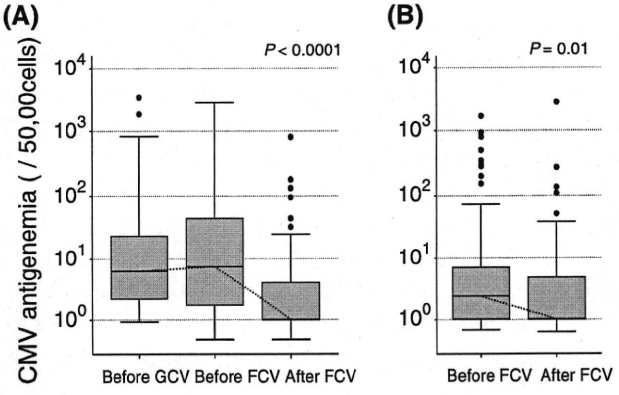

Fig. 3 Change in CMV antigenemia due to foscarnet therapy. The levels of antigenemia before ganciclovir, before foscarnet, and after foscarnet are box plotted. A significant decrease in antigenemia due to foscarnet treatment was observed both (**a**) for patients who had received prior ganciclovir treatment and (**b**) for those who had not

The responses to foscarnet according to the 5 dose categories are summarized in Fig. 4. The symptoms of CMV disease improved in around 50% of patients in every dose category. In the CMV disease patients the response rate of

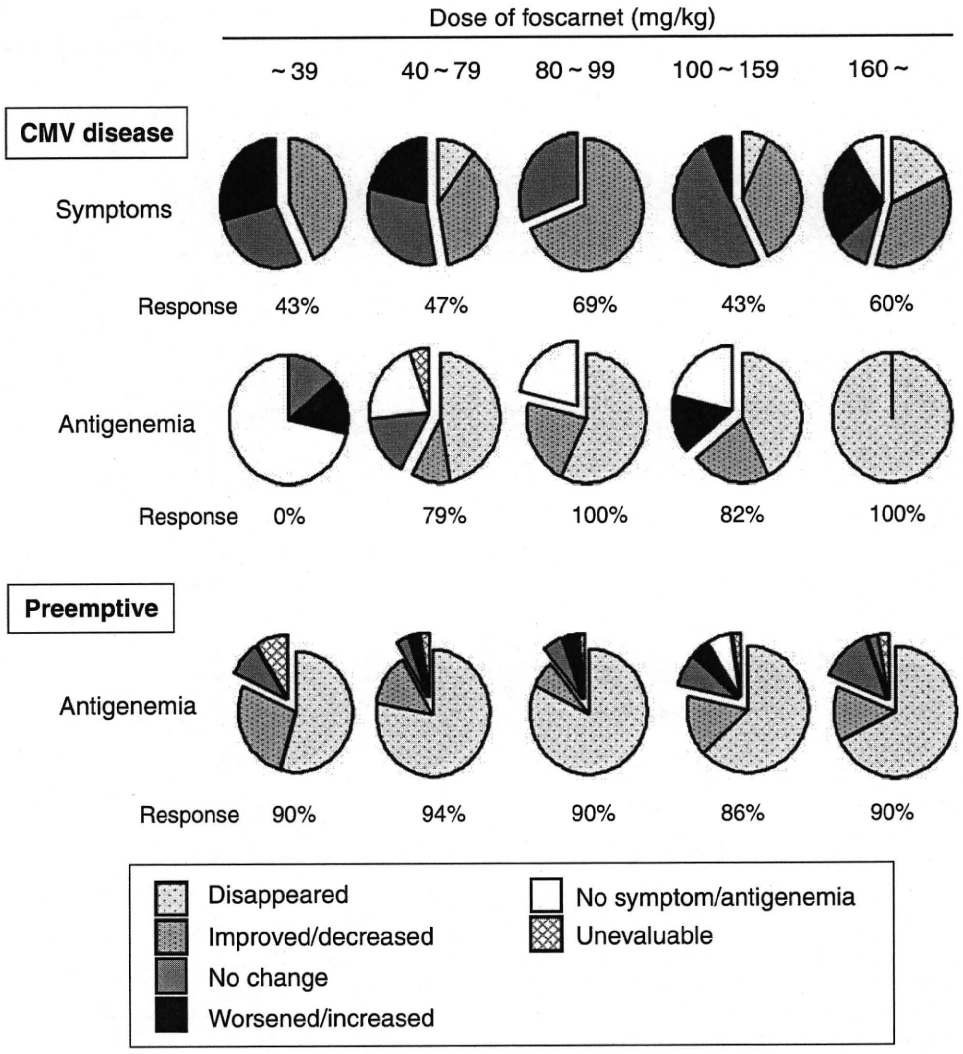

Fig. 4 Response to foscarnet according to 5 dose categories. The number of patients from the CMV disease group was 7 in the <39 mg/kg group, 19 in the 40–79 mg/kg group, 14 in the 80–99 mg/kg group, 14 in the 100–159 mg/kg group, and 11 in the 160 mg/kg or higher group, and those of the preemptive group were 11, 81, 73, 46, and 37, respectively. The response rate was around 50% for symptoms of CMV disease and was generally higher for antigenemia

antigenemia was significantly lower for those received foscarnet <40 mg/kg ($P = 0.01$).

3.5 Survival

The overall survival of all patients who received foscarnet was 34% at a median follow-up of 3 years (Fig. 5a). Patients with CMV disease showed significantly lower survival than those who received preemptive or prophylactic therapy (Fig. 5b, $P = 0.0004$). No significant difference in prognosis was found between the patients with and without preceding other anti-viral agents ($P = 0.21$). A total of 170 patients died, and the main causes of death were disease recurrence in 47, bacterial sepsis in 27, acute/chronic graft-versus-host disease in 25, and fungal infection in 10. The cumulative incidence of transplant-related mortality at 1 year was 30% (95% confidence interval 25–35%). Three patients eventually died of CMV disease, and the cumulative incidence of CMV-associated death at 1 year was 1.0% (95% confidence interval 0.3–2.6%).

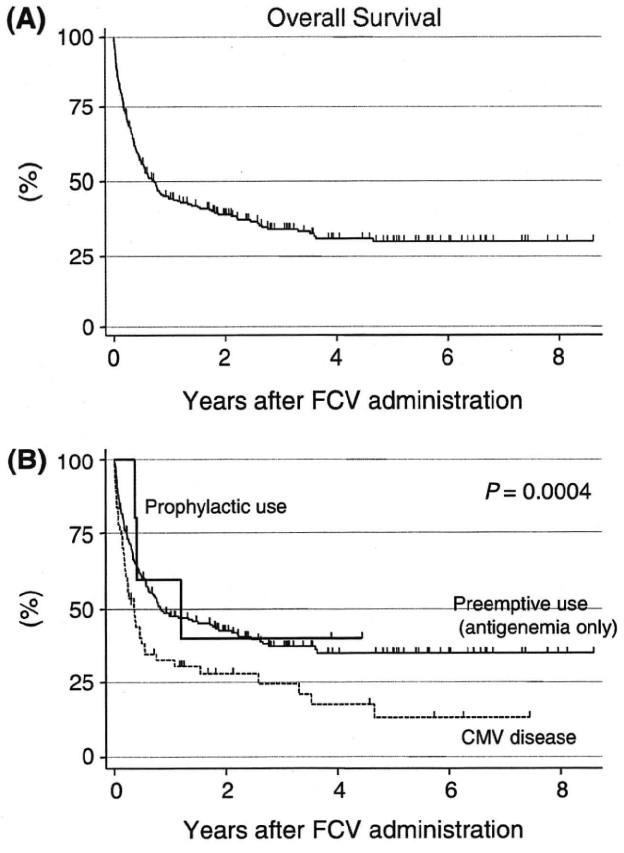

Fig. 5 Overall survival (OS) of patients who received foscarnet therapy. **a** The 3-year OS was 34%. **b** The prognosis of patients with CMV disease was significantly poorer than those of patients who had received preemptive or prophylactic use ($P = 0.0004$)

3.6 Adverse events

Adverse events (irrespective of causal association) of NCI-CTCAE grade 3 or higher are listed in Table 3. The most common adverse event was electrolyte abnormalities, which occurred in 35 patients (11%). The other major toxic events included neutropenia in 27 patients, thrombocytopenia in 26 patients, and bone marrow dysfunction in 11 patients. Renal and hepatic damage developed in 11 and 10 patients, respectively. Adverse events associated with foscarnet included neutropenia in 5 patients; electrolyte abnormalities in 4 patients; thrombocytopenia, renal dysfunction and sensory disturbance in 2 patients each; and bone marrow dysfunction in 1 patient. No patient died of an adverse reaction associated with foscarnet. The total number of patients who developed a grade 3 adverse reaction or higher was 56 (28%) in the patients who received prior ganciclovir and 21 (17%) in those who did not ($P = 0.03$). The rate of adverse events did not differ among the 5 dose categories (Table 4). The duration of foscarnet medication was not different between patients who developed adverse event of grade 3 or more (median 16 days, range 2–121) and those did not (median 20 days,

Table 3 Adverse events during foscarnet treatment

	Prior GCV $N = 198$		No prior GCV $N = 122$		Total $N = 320$	
	N	%	*N*	%	*N*	%
Graft failure	2	1.0	2	1.6	4	1.3
Neutropenia	19	9.6	8	6.6	27	8.4
Grade 3	7	3.5	2	1.6	9	2.8
Grade 4	12	6.1	6	4.9	18	5.6
Thrombocytopenia	19	9.6	7	5.7	26	8.1
Grade 3	6	3.0	0	0.0	6	1.9
Grade 4	13	6.6	7	5.7	20	6.3
BM dysfunction	7	3.5	3	2.5	10	3.1
Grade 3	4	2.0	1	0.8	5	1.6
Grade 4	3	1.5	2	1.6	5	1.6
Renal damage	6	3.0	5	4.1	11	3.4
Grade 3	4	2.0	5	4.1	9	2.8
Grade 4	2	1.0	0	0.0	2	0.6
Electrolyte abnormality	27	13.6	8	6.6	35	10.9
Grade 3	20	10.1	7	5.7	27	8.4
Grade 4	7	3.5	1	0.8	8	2.5
Neurological	3	1.5	1	0.8	4	1.3
Grade 3	3	1.5	1	0.8	4	1.3
Grade 4	0	0.0	0	0.0	0	0.0
Liver damage	9	4.5	1	0.8	10	3.1
Grade 3	7	3.5	0	0.0	7	2.2
Grade 4	2	1.0	1	0.8	3	0.9

BM bone marrow

Table 4 Adverse effects according to foscarnet dose

Dose level (mg/kg)	0–39 $N = 18$ (%)	40–79 $N = 106$ (%)	80–99 $N = 88$ (%)	100–159 $N = 60$ (%)	160– $N = 48$ (%)	Total $N = 320$ (%)
Any grade 3 or higher	33	23	17	25	35	24
Grade 3 or higher, possibly by foscarnet	28	12	13	17	17	15
Grade 3 or higher, definitely by foscarnet	0	2.8	3.4	8.3	6.3	4.4

range 1–322, $P = 0.50$). The difference was not evident for patients with possible and definite association with foscarnet ($P = 0.84$ and $P = 0.22$, respectively). When the adverse events were compared between HLA-matched and -mismatched transplant, the rates were significantly higher in the HLA-matched transplant. Any grade 3 or more toxicity was developed in 36 of 108 HLA-matched and 33 of 160 HLA-mismatched transplant ($P = 0.02$). Of these, 31 and 24, respectively, were possibly due to foscarnet use (29 vs. 15%, $P = 0.009$).

4 Discussion

The present study demonstrated that foscarnet is effective for patients with CMV infection who are not suitable for ganciclovir therapy. Sixty percent of the patients had a history of prior ganciclovir, but had demonstrated problems of ineffectiveness and/or adverse reactions. The remaining 40% had poor bone marrow function, and therefore foscarnet had been selected as the up-front use. In both situations, most of the patients were preemptively treated, and prophylactic use was seen in <2% of cases in our series.

The initial dose of foscarnet had two convergent doses, which were 90 and 180 mg/kg. The former corresponds to the maintenance dose, and the latter is the initial dose which was used in most prospective studies [18, 19]. The dose of foscarnet was significantly higher in patients with secondary therapy. This might have resulted from a higher number of more severe patients with CMV infection being present in the secondary therapy group. On the other hand, no dosage differences were found between the various purpose groups (preemptive/prophylactic/treatment). The lack of a correlation between foscarnet dose and creatinine clearance suggested that foscarnet was used irrespective of the renal function of the patient.

The most important adverse reaction of foscarnet was previously described as renal damage including electrolyte abnormalities. In that study, one-third of patients developed renal insufficiency and/or electrolyte disturbance [15]. However, a later study showed that these adverse events occurred less frequently [19]. In our series of patients, electrolyte abnormalities were recognized in 11% of patients, and renal insufficiency was found in no >3% of patients, which was consistent with the findings in the literature [24]. Thus, foscarnet seems to be a safer drug than was initially predicted.

In the preemptive use of foscarnet, >80% of patients showed CMV antigenemia disappearance in both the initial and secondary therapy groups. Foscarnet was highly effective in this setting, but its efficacy was decreased in CMV disease. The efficacy of foscarnet did not correlate with its dose, which was contradictory to a previous dose-finding study [25]. Our findings suggest a need to explore appropriate therapeutic strategies for this agent. Recently, "low-dose" administration of foscarnet at 60 mg/kg/day has been reported to be effective for CMV preemptive treatment [26, 27], which could be an option for future clinical trials. A prospective trial comparing ganciclovir alone and a combination of ganciclovir and foscarnet (half doses of both) was performed for HSCT and organ transplant patients [28]. The efficacy was equivalent for both arms, but adverse events were more frequent in the foscarnet combined arm.

In conclusion, our study shows that foscarnet is a safe and effective agent for treating CMV antigenemia after allogeneic HSCT. It remains to be determined how CMV infections should be treated, as well as how to improve the survival of affected patients.

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Hospital; Mie University; Suzuka Kaisei Hospital; Kyoto University; Social Insurance Kyoto Hospital; Kyoto City Hospital; Kyoto Katsura Hospital; Osaka Medical Center For Cancer And Cardiovascular Diseases; Kinki University; Hyogo College of Medicine; Osaka City University; Matsushita Memorial Hospital; Rinku General Medical Center Izumisano Municipal Hospital; Osaka Medical College; Kansai Medical University Hirakata Hospital; Hyogo Cancer Center; Shimane Prefectural Central Hospital; Kurashiki Central Hospital; Okayama University; National Hospital Organization Kure Medical Center; Yamaguchi University; Tokushima University; Kyushu University; Hamanomachi Hospital; Nagasaki University; Sasebo City General Hospital; National Hospital Organization Nagasaki Medical Center; National Hospital Organization Kumamoto Medical Center; Oita University.

References

1. Stocchi R, Ward KN, Fanin R, Baccarani M, Apperley JF. Management of human cytomegalovirus infection and disease after allogeneic bone marrow transplantation. Haematologica. 1999;84:71–9.
2. Boeckh M. Current antiviral strategies for controlling cytomegalovirus in chematopoietic stem cell transplant recipients: prevention and therapy. Transpl Infect Dis. 1999;1:165–78.
3. Boeckh M, Nichols WG, Papanicolaou G, Rubin R, Wingard JR, Zaia J. Cytomegalovirus in hematopoietic stem cell transplant recipients: current status, known challenges, and future strategies. Biol Blood Marrow Transplant. 2003;9:543–58.
4. Yanada M, Yamamoto K, Emi N, Naoe T, Suzuki R, Taji H, Iida H, Shimokawa T, Kohno A, Mizuta S, Maruyama F, Wakita A, Kitaori K, Yano K, Hamaguchi M, Hamajima N, Morishima Y, Kodera Y, Sao H, Morishita Y. Cytomegalovirus antigenemia and outcome of patients treated with pre-emptive ganciclovir: retrospective analysis of 241 consecutive patients undergoing allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant. 2003;32:801–7.
5. Biron K. Antiviral drugs for cytomegalovirus disease. Antiviral Res. 2006;71:154–63.
6. Goodrich JM, Mori M, Gleaves CA, Du Mond C, Cays M, Ebeling DF, Buhles WC, DeArmond B, Meyers JD. Early treatment with ganciclovir to prevent cytomegalovirus disease after allogeneic bone marrow transplantation. N Engl J Med. 1991; 325:1601–7.
7. Goodrich JM, Bowden RA, Fisher L, Keller C, Schoch G, Meyers JD. Ganciclovir prophylaxis to prevent cytomegalovirus disease after allogeneic marrow transplant. Ann Intern Med. 1993;118:173–8.
8. Winston DJ, Ho WG, Bartoni K, Du Mond C, Ebeling DF, Buhles WC, Champlin RE. Ganciclovir prophylaxis of cytomegalovirus infection and disease in allogeneic bone marrow transplant recipients. Results of a placebo-controlled, double-blind trial. Ann Intern Med. 1993;118:179–84.
9. Salzberger B, Bowden RA, Hackman RC, Davis C, Boeckh M. Neutropenia in allogeneic marrow transplant recipients receiving ganciclovir for prevention of cytomegalovirus disease: risk factors and outcome. Blood. 1997;90:2502–8.
10. Crumpacker CS. Ganciclovir. N Engl J Med. 1996;335:721–9.
11. Centers for Disease Control and Prevention, Infectious Diseases Society of America, American Society of Blood and Marrow Transplantation. Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. Biol Blood Marrow Transplant. 2000;6(Suppl 6):7–83.
12. Baldanti F, Lurain N, Gerna G. Clinical and biologic aspects of human cytomegalovirus resistance to antiviral drugs. Hum Immunol. 2004;65:403–9.
13. Balfour HH Jr. Antiviral drugs. N Engl J Med. 1999;340: 1255–68.
14. Ippoliti C, Morgan A, Warkentin D, van Besien K, Mehra R, Khouri I, Giralt S, Gajewski J, Champlin R, Andersson B, Przepiorka D. Foscarnet for prevention of cytomegalovirus infection in allogeneic marrow transplant recipients unable to receive ganciclovir. Bone Marrow Transplant. 1997;20:491–5.
15. Ordemann R, Naumann R, Geissler G, Kroschinsky F, Bornhäuser M, Schwerdtfeger R, Ehninger G. Foscarnet: an alternative for cytomegalovirus prophylaxis after allogeneic stem cell transplantation? Ann Hematol. 2000;79:432–6.
16. Reusser P, Gambertoglio JG, Lilleby K. Phase I-II trial of foscarnet for prevention of cytomegalovirus infection in autologous and allogeneic transplant recipients. J Infect Dis. 1992;166: 473–9.
17. Bacigalupo A, Tedone E, van Lint MT, Trespi G, Lonngren M, Sanna MA, Moro F, Frassoni F, Occhini D, Gualandi F, et al. CMV prophylaxis with foscarnet in allogeneic bone marrow transplant recipients at high risk of developing CMV infection. Bone Marrow Transplant. 1994;13:783–8.
18. Moretti S, Zikos P, van Lint MT, Tedone E, Occhini D, Gualandi F, Lamparelli T, Mordini N, Berisso G, Bregante S, Bruno B, Bacigalupo A. Foscarnet vs ganciclovir for cytomegalovirus (CMV) antigenemia after allogeneic hemopoietic stem cell transplantation (HSCT): a randomised study. Bone Marrow Transplant. 1998;22:175–80.
19. Reusser P, Einsels H, Lee J, Volin L, Rovira M, Engelhard D, Finke J, Cordonnier C, Link H, Ljungman P. Randomized multicenter trial of foscarnet versus ganciclovir for preemptive therapy of cytomegalovirus infection after allogeneic stem cell transplantation. Blood. 2002;99:1159–64.
20. Matsumura T, Narimatsu H, Kami M, Yuji K, Kusumi E, Hori A, Murashige N, Tanaka Y, Masuoka K, Wake A, Miyakoshi S, Kanda Y, Taniguchi S. Cytomegalovirus infections following umbilical cord blood transplantation using reduced intensity conditioning regimens for adult patients. Biol Blood Marrow Transplant. 2007;13:577–83.
21. Atsuta Y, Suzuki R, Yoshimi A, Gondo H, Tanaka J, Hiraoka A, Kato K, Tabuchi K, Tsuchida M, Morishima Y, Mitamura M, Kawa K, Kato S, Nagamura T, Takanashi M, Kodera Y. Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP system. Int J Hematol. 2007;86:269–74.
22. Kanda Y, Mineishi S, Saito T, Seo S, Saito A, Suenaga K, Ohnishi M, Niiya H, Nakai K, Takeuchi T, Kawahigashi N, Shoji N, Ogasawara T, Tanosaki R, Kobayashi Y, Tobinai K, Kami M, Mori S, Suzuki R, Kunitoh H, Takaue Y. Pre-emptive therapy against cytomegalovirus (CMV) disease guided by CMV antigenemia assay after allogeneic haematopoietic stem cell transplantation: a single-center experience in Japan. Bone Marrow Transplant. 2001;27:437–44.
23. Mori T, Mori S, Kanda Y, Yakushiji K, Mineishi S, Takaue Y, Gondo H, Harada M, Sakamaki H, Yajima T, Iwao Y, Hibi T, Okamoto S. Clinical significance of cytomegalovirus (CMV) antigenemia in the prediction and diagnosis of CMV gastrointestinal disease after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant. 2004;33:431–4.
24. Ljungman PG, Oberg G, Aschan J, Ehrnst A, Lönnqvist B, Pauksen K, Sulila P. Foscarnet for pre-emptive therapy of CMV infection detected by a leukocyte-based nested PCR in allogeneic bone marrow transplant patients. Bone Marrow Transplant. 1996; 18:565–8.
25. Bregante S, Bertilson S, Tedone E, Van Lint MT, Trespi G, Mordini N, Berisso G, Gualandi F, Lamparelli T, Figari O, Benvenuto F, Raiola AM, Bacigalupo A. Foscarnet prophylaxis of cytomegalovirus infections in patients undergoing allogeneic

bone marrow transplantation (BMT): a dose-finding study. Bone Marrow Transplant. 2000;26:23–9.
26. Narimatsu H, Kami M, Kato D, Matsumura T, Murashige N, Kusumi E, Yuji K, Hori A, Shibata T, Masuoka K, Wake A, Miyakoshi S, Morinaga S, Taniguchi S. Reduced dose of foscarnet as preemptive therapy for cytomegalovirus infection following reduced-intensity cord blood transplantation. Transpl Infect Dis. 2007;9:11–5.
27. Wang H, Zhu L, Xue M, Liu J, Guo Z. Low-dose foscarnet preemptive therapy for cytomegalovirus viremia after haploidentical bone marrow transplantation. Biol Blood Marrow Transplant. 2009;15:519–20.
28. Mattes FM, Hainsworth EG, Geretti AM, Nebbia G, Prentice G, Potter M, Burroughs AK, Sweny P, Hassan-Walker AF, Okwuadi S, Sabin C, Amooty G, Brown VS, Grace SC, Emery VC, Griffiths PD. A randomized, controlled trial comparing ganciclovir to ganciclovir plus foscarnet (each at half dose) for preemptive therapy of cytomegalovirus infection in transplant recipients. J Infect Dis. 2004;189:1355–61.

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Allogeneic stem cell transplantation for adult Philadelphia chromosome negative acute lymphocytic leukemia: comparable survival rates but different risk factors between related and unrelated transplantation in first complete remission

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Allogeneic stem cell transplantation for adult Philadelphia chromosome–negative acute lymphocytic leukemia: comparable survival rates but different risk factors between related and unrelated transplantation in first complete remission

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To identify factors to improve the outcomes of related and unrelated allogeneic stem cell transplantations (allo-SCT) for Philadelphia chromosome–negative acute lymphocytic leukemia (Ph$^-$ ALL) in the first complete remission (CR1), we retrospectively analyzed 1139 Ph$^-$ ALL patients using the registry data, particularly the details of 641 patients transplanted in CR1. Overall survival was significantly superior among patients transplanted in CR1, but no significant difference was observed between related and unrelated allo-SCTs (related vs unrelated: 65% vs 62% at 4 years, respectively; $P = .19$). Among patients transplanted in CR1, relapse rates were significantly higher in related allo-SCT compared with unrelated allo-SCT, and multivariate analysis demonstrated that less than 6 months from diagnosis to allo-SCT alone was associated with relapse. On the other hand, nonrelapse mortality (NRM) was significantly higher in unrelated allo-SCT compared with related allo-SCT, and multivariate analysis demonstrated that 10 months or longer from diagnosis to allo-SCT, human leukocyte antigen mismatch, and abnormal karyotype were associated with NRM. In conclusion, our study showed comparable survival rates but different relapse rates, NRM rates, and risk factors between related and unrelated allo-SCTs. After a close consideration of these factors, the outcome of allo-SCT for adult Ph$^-$ ALL in CR1 could be improved. (*Blood.* 2010;116(20):4368-4375)

Introduction

The indication of allogeneic stem cell transplantation (allo-SCT) for Philadelphia chromosome–negative acute lymphocytic leukemia (Ph$^-$ ALL) is still controversial.[1,2] As for related allo-SCT, one prospective study suggested that related allo-SCT for Ph$^-$ ALL in first complete remission (CR1) could provide the most potent antileukemic therapy and considerable survival benefits.[3] As for unrelated allo-SCT, the largest retrospective study of Ph$^-$ ALL patients in CR1 showed worse overall survival (OS) rates because of higher incidences of nonrelapse morality (NRM) than those in related allo-SCT,[4] whereas another reported that there were no differences in OS rates and NRM rates between related and unrelated allo-SCTs for adult ALL in CR1.[5] These data indicated that unrelated allo-SCT could also be a treatment option for adult Ph$^-$ ALL patients in CR1 if NRM rates were low enough, although it is not yet routinely performed.

Although the analyses of the outcome of allo-SCT alone have some biases, such as excluding death during chemotherapy, and there may be potential differences in the baseline characteristics of patients between related and unrelated allo-SCTs, the comparison of transplantation outcomes and risk factors between related and unrelated allo-SCTs for adult Ph$^-$ ALL could indicate strategies to improve transplantation outcomes for this disease. We particularly focused on allo-SCT in CR1 because this is the area of controversy.

Methods

Collection of data and data sources

The recipients' clinical data were provided by the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and the Japan Marrow Donor Program (JMDP). Both JSHCT and JMDP collect recipients' clinical data at 100 days after allo-SCT. The patient's data on survival, disease status, and long-term complications, including chronic graft-versus-host disease (GVHD) and second malignancies, are renewed annually by follow-up forms. More than 99% of unrelated allo-SCT in Japan was captured in the JMDP database, and approximately 75% of related allo-SCT was captured in the JSHCT database. This study was approved by the data management committees of JSHCT and JMDP. Informed consent was obtained from both recipients and donors in accordance with the Declaration of Helsinki.

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