Allogeneic bone marrow transplantation for hepatocellular carcinoma: hepatocyte growth factor suppresses graft-vs.-host disease

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Hepatocellular carcinoma (HCC) is a highly lethal malignancy although several therapeutic strategies are available for this disease. A novel therapeutic strategy is urgently required to improve the prognosis of HCC. Wada et al. (42) previously reported a clinicopathological study in which it was found that some patients with HCC had strong intratumoral lymphocyte infiltration, which correlated with a lower recurrence of disease and better survival compared with patients with weak intratumoral lymphocyte infiltration. This finding suggests that immunotherapy may be a viable form of therapy for HCC. Allogeneic bone marrow transplantation (BMT) is known to induce powerful graft-vs.-tumor (GVT) effects and is performed on patients with hematological malignancies, including myeloid and lymphoid leukemias, lymphomas, and multiple myeloma (17, 41, 43). It has been reported that allogeneic BMT-mediated GVT effects are also effective for solid tumors, such as metastatic breast carcinoma and metastatic renal cell carcinoma (3, 4, 40). Therefore, we hypothesized that allogeneic BMT can induce GVT effects on HCC and improve the survival of HCC patients.

The GVT effects are initiated by contaminating donor T cells in the bone marrow, but these cells also induce graft-vs.-host disease (GVHD) (25). Acute GVHD is characterized by hematopoietic dysfunction, immunosuppression, and tissue injuries in the skin, liver, and intestinal mucosa (5, 38). Several approaches for preventing acute GVHD have focused on the elimination of donor T cells from the graft or the use of immunosuppressive agents, such as FK506. However, these approaches result in a poorer prognosis for allogeneic BMT patients because these methods reduce the GVT activity induced by donor T cell responses to host antigen (12).

Hepatocyte growth factor (HGF) was originally identified and cloned as a potent mitogen for hepatocytes (7, 22). It has mitogenic, motogenic, and morphogenic effects on various epithelial tissues, including the liver, kidneys, lungs, and intestines (21, 45). We recently demonstrated that HGF gene transduction can reduce acute GVHD while preserving the GVT effects in a leukemia animal model (13). This study is the first to demonstrate an antitumor effect of allogeneic BMT on HCC and assessed whether HGF might preserve the GVT effect but ameliorate the GVHD in a mouse model system.

MATERIALS AND METHODS

Cell lines and cell cultures. The mouse HCC cell line, Hepal-a, derived from a hepatoma in a C57L mouse (H-2b), EL4, derived from a lymphoma cell line in a C57BL/6 mouse (H-2b) and YAC-1, a lymphoma cell line that was induced by inoculation of the Moloney murine leukemia virus into a newborn A/Sn mouse, were grown in RPMI medium with 10% fetal calf serum.

Cell proliferation assay. Wells of 96-well microtiter plates were seeded with 4 × 10³ Hepal-a cells in 0.2 ml of medium containing various amounts of recombinant HGF (1, 10, or 20 ng/ml). Cells were harvested at 1, 3, or 5 days and counted by a crystal violet staining procedure (1).

Animals. C3H/HeJ (C3H, H-2b) mice were used as the BMT donors and (C3H × C57BL/6)F1 (B6C3F1, H-2b) mice were used as the BMT recipients. All mice were 8–12 wk old and were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan). The mice were maintained in a pathogen-free facility at Hyogo College of Medicine.
BMT with empty HVJ (group 2) to receive different treatments: all mice 1 wk after injection. The mice then were divided into six groups

- Group 1: (no treatment)
- Group 2: (TBI)
- Group 3: (TBI+BMT)
- Group 4: (TBI+BMT+HVJ-empty)
- Group 5: (TBI+BMT+HVJ-HGF)
- Group 6: (TBI+BMT+FK506)

Fig. 1. Experimental protocol. All B6C3F1 mice were given subcutaneous injections in the back with 5 × 10^6 Hepa1-a cells on day 0. Total body irradiation (TBI) was performed from an X-ray source (9 Gy) on day 7. Bone marrow transplantation (BMT) was performed by the intravenous injection of C3H bone marrow cells (5 × 10^6) and spleen cells (2 × 10^7) on day 7. Hepatocyte growth factor (HGF) gene transduction was performed on days 4, 11, 18, and 25. FK506 (5 mg/kg) was administrated daily from day 7 to day 28. Tumor size was measured once a week. HVJ, Hemagglutinating virus of Japan.

Medicine (Nishinomiya, Hyogo, Japan). The animal experiments were performed in accordance with the guidelines of the National Institutes of Health (Bethesda, MD), as specified by the animal care policy of Hyogo College of Medicine. All experimental protocols for animal research in the present study were submitted to and approved by the animal experimental review committee at Hyogo College of Medicine.

**BMT.** C3H/HeJ donor mice were killed, and femurs and spleens were removed. Bone marrow cells were harvested by flushing femurs with RPMI 1640 medium (GIBCO) via a 26-gauge needle and syringe. Spleen cells were harvested by smashing spleens on stainless steel mesh. Bone marrow cells and spleen cells were incubated for 5 min in Tris-NH4Cl to lyse red blood cells, and resulting cells were washed twice with PBS. B6C3F1 mice were exposed to total body irradiation (TBI) from an X-ray source (9 Gy at a dose rate of 0.5 Gy/min), after which the bone marrow cells (5 × 10^6) plus spleen cells (2 × 10^7) from C3H/HeJ donor mice were injected via the tail vein (12).

**HGF gene transduction.** Human HGF cDNA (2.2 kb) was inserted into the EcoRI and NotI sites in the plasmid pUC-SC-Rα under the control of the SRα promoter (29). Hemagglutinating virus of Japan (HVJ) liposomes were used as a carrier system. HVJ liposomes containing plasmid DNA and high-mobility group 1 nonhistone chromosomal protein purified from calf thymus were constructed as described previously (11). Briefly, phosphatidylserine, phosphatidylcholine, and cholesterol were mixed at a weight ratio of 1:4.8:2. This lipid mixture (1 mg) plus plasmid DNA (20 to 40 μg), which had previously been complexed with 6–12 μg of high-mobility group 1, was sonicated to form liposomes and then mixed with ultraviolet-irradiated HVJ. Excess free virus was subsequently removed from the HVJ liposomes by sucrose density gradient centrifugation. B6C3F1 mice were injected into the gluteal muscle with the HVJ liposomes containing 8 μg of a human HGF expression vector (HGF-HVJ liposomes). The gene transfer was repeated once a week for 4 wk.

**Experimental animal models.** B6C3F1 mice were used for a subcutaneous tumor model. We injected 5 × 10^6 of Hepa1-a cells into one area of the flank of each mouse on day 0. A small induration formed in all mice 1 wk after injection. The mice then were divided into six groups to receive different treatments: group 1: no further treatment (n = 19); group 2: TBI (n = 15); group 3: TBI + BMT (n = 20); group 4: TBI + BMT with empty HVJ (n = 12); group 5: TBI + BMT + HGF gene transduction (n = 30); group 6: TBI + BMT + FK506 (n = 12). BMT was performed on day 7. HGF gene transduction was given on days 4, 11, 18, and 25. FK506, a representative immunosuppressive agent, was provided by Fujisawa Pharmaceutical (Osaka, Japan). FK506 in a carrier solvent of polyethylene castor oil and ethanol was diluted into normal saline. The FK506 solution (5 mg/kg) was subcutaneously administrated daily (in a final volume of 0.1 ml per mouse) from day 7 to day 28. The tumor size was measured once a week with calipers. Volumes of tumors were determined by the formula volume = width^2 × length × 0.52. The time schedule is shown in Fig. 1.

**Histopathology.** Histopathological changes were examined 3 wk after BMT (day 28). Tissues were fixed in 10% buffered formalin and embedded in paraffin. The sections were stained with hematoxylin and eosin and were examined by light microscopy. The microscopic scoring of the hematoxylin and eosin staining (H-E staining) was carried out in a blind fashion by two researchers who did not cooperate this study. The length of villi in the small intestine was measured with a calibrated lens to study at least 20 complete and straight villi per slide in five mice that were randomly selected from each group. The number of inflammatory cells around intrahepatic bile duct was also determined in a blind fashion. Five intrahepatic bile...
ducts per slide in five mice that were randomly selected in each group and two researchers independently counted inflammatory cells.

Immunohistochemical analysis. To investigate whether BMT actually achieved donor T cells infiltration in solid HCC subcutaneous tumor of mice, BMT was performed for five mice 14 days after Hepa1-a injection when tumor volume reached ~200 mm³. Five mice were given TBI as a control. Mice were killed and subcutaneous tumors were removed for immunohistochemical analysis 2 wk after BMT or TBI. For enzyme antibody staining, tumors were frozen in optimal cutting temperature compound and were cut into 5-μm slices, mounted on microscope slides, and fixed in pure acetone. The slides were hydrated with PBS for 15 min. Sections were incubated in 0.3% H₂O₂ for 30 min at room temperature, then for 15 min at room temperature in avidin-biotin blocking agent (Vector Laboratories). After being blocked with nonspecific staining blocking reagent (DAKO) for 15 min at room temperature, the slides were incubated overnight at 4°C with a primary antibody (biotinylated anti-H-2Kk, anti-H-2Kb, anti-CD4, and anti-CD8a mAbs; 1:30 dilution. BD Biosciences Pharmingen, San Jose, CA). The slides were incubated with horseradish peroxidase-labeled streptavidin and reacted with diaminobenzidine tetrachloride (33).

For double immunofluorescent staining, frozen sections were fixed in pure acetone, then incubated for 15 min at room temperature in avidin-biotin blocking agent. After being blocked with nonspecific staining blocking reagent for 15 min at room temperature, the lymphocytes were identified by staining with a biotinylated anti-CD4 mAb and a H-2Kk mAb, and FITC-labeled anti-CD8 mAb and a H-2Kb mAb at 4°C overnight and visualized with Alexa Fluor 488 or Alexa Fluor 546 (Molecular Probes, Invitrogen, Carlsbad, CA). Sections for fluorescent staining were analyzed with a confocal laser scanning microscope (LSM510; Carl Zeiss, Jena, Germany) (6).

Flow cytometry. Bone marrow cell or spleen cell suspensions derived from the donor or recipient mice were prepared in PBS containing 1% FCS and 0.1% sodium azide. Cells were incubated with an anti-Fc receptor mAb (2.4G2) for 10 min at 4°C, and then incubated with a FITC-conjugated mAb and a phycoerythrin-conjugated mAb for 30 min. Stained cells were washed twice, resuspended, and analyzed by means of a FACSscan (Becton Dickinson, Mountain View, CA). Anti-Fc receptor (2.4G2) mAb, FITC-conjugated anti-mouse H-2Kb (clone AF6-88.5) mAb, and phycoerythrin-conjugated anti-mouse H-2Kk (clone 36-7-5) were purchased from BD Biosciences Pharmingen.

Fig. 3. Surface major histocompatibility complex (MHC) class I expression on spleen cells (top) and bone marrow cells (bottom). At 14 days after BMT, MHC class I expression on the spleen cells and bone marrow cells of the recipient mice was examined by flow cytometry. Spleen cells and bone marrow cells completely changed from recipient type (H-2Kk/H-2Kb) to donor type (H-2Kk/H-2Kb).
ELISA for HGF and IFN-γ. The human HGF concentration in the plasma was measured by an ELISA using an anti-human HGF mAb (Institute of Immunology, Tokyo, Japan). The mouse IFN-γ concentration in the serum was measured by ELISA using an anti-mouse IFN-γ mAb (Genzyme Pharmaceuticals, Cambridge, MA). These assays were performed according to the manufacturer’s protocol.

51Cr release assay. Effector cytotoxic T lymphocyte (CTL) activity was tested by the 51Cr release assay as described previously (14). Briefly, freshly harvested spleen cells from BMT recipients (B6C3F1) were cocultured with irradiated (20Gy) B6C3F1 spleen cells in vitro. After 3 days of culture, the cells were harvested and the CTL activity was examined on the basis of the lysis of Hepa1-a or EL4 lymphoma cells derived from C57BL/6 (H-2b, as a positive control) in a 4-h 51Cr release assay. Spleen cells were added at varying effector-target cell (E/T) ratios and incubated for 4 h with either Hepa1-a or EL4 lymphoma cells labeled with 100 μCi (3.7 MBq) of 51Cr. Effector cells were tested in triplicate, and the percentage of lysis was calculated according to the following formula: [(sample cpm − spontaneous cpm)/(maximum cpm − spontaneous cpm)] × 100%. Results are shown as the mean percentage of lysis at a given E/T ratio. To assess the contribution of natural killer (NK) cells to tumor suppression, YAC-1 cells that are sensitive to the action of NK cells were used following the same procedure.

Statistical analysis. Statistical significance was evaluated by ANOVA followed by Tukey’s post hoc test using SSPS II for Windows (SSPS Japan, Tokyo, Japan) to compare the multiple groups. Differences were considered to be statistically significant at the level of \( P \leq 0.05 \).

RESULTS

Effects of HGF on HCC cell proliferation in vitro. The effect of HGF on Hepa1-a cell proliferation was assessed in vitro. Hepa1-a cells were incubated with various amounts of recombinant HGF. No significant effect was seen on cell proliferation (Fig. 2).
Fig. 6. Effect of HGF on liver injury. H-E staining of the liver (A–F, original magnification ×40, insets ×100). A: no treatment. B: TBI only. Inflammatory cells infiltrating around the intrahepatic bile duct were seen in mice that received TBI + BMT and TBI + BMT with HVJ empty (C and D, arrows). Cellular infiltration was improved by HGF gene transfer or FK506 administration (E and F).

Fig. 7. A: length of villi in the small intestine was measured with a calibrated lens. Twenty complete and straight villi per slide were randomly selected and 2 researchers independently measured the villi in 5 mice from all groups. B: number of infiltrating cells around the intrahepatic bile duct. Five intrahepatic bile ducts were randomly selected and 2 researchers independently counted the total number of stained infiltrating cells in 5 mice from all groups. *P < 0.05.
Donor cell engraftment after BMT. To evaluate donor cell engraftment after BMT, surface MHC class I expression on spleen cells and bone marrow cells from the BMT mice was analyzed by FACScan. Staining of recipient cells gave unimodal-positive profile by staining with both anti-H-2Kk and anti-H-2Kb Abs when compared with negative controls. In contrast, at 14 days after BMT, cells from BMT mice gave positive profile by staining with anti-H-2 Kk Ab but negative profile with anti-H-2Kb Ab, compatible to the staining of donor cells. These results indicated that both the bone marrow cells and the spleen cells in the recipients completely changed to the donor type (Fig. 3).

Growth suppression in subcutaneous Hepa1-a-cell tumors. 5 × 10^6 of Hepa1-a cells were injected into one area in the flank of each B6C3F1 mouse on day 0. At 7 days after tumor cell injection, a small induration formed in all mice. BMT was performed in four groups: group 3, TBI + BMT (n = 20); group 4, TBI ± BMT with empty HVJ (n = 12); group 5: TBI + BMT + HGF gene transduction (n = 30); or group 6, TBI + BMT + FK506 (n = 12). The growth suppression of the tumors caused by BMT is shown in Fig. 4. The mean volume of tumors at day 28 was as follows: group 1, 1,263 mm^3; group 2, 980 mm^3; group 3, 104 mm^3; group 4, 124 mm^3; group 5, 96 mm^3; group 6, 336 mm^3. BMT significantly suppressed tumor growth (P < 0.05). However, among the four groups that underwent BMT, this suppressive effect was weaker in group 6 (TBI + BMT + FK506) mice that were administered immunosuppressive agent (FK506) instead of HGF gene transduction. Significant difference was not seen between group 2 (TBI only) and group 6.

Effect of HGF on intestinal and liver injury caused by GVHD. We previously reported that repeated transduction of the human HGF gene into skeletal muscle resulted in a sustained high serum concentration of human and endogenous rat or mouse HGF (19, 24, 39). Human and mouse HGF both were detected by ELISA in the sera from mice transduced with the human HGF gene. The total concentration of HGF (human and mouse) was 0.18 ng/ml before injection and increased to between 1.12 and 1.43 ng/ml over the 7 days after transfection. We also reported that HGF gene transduction reduced tissue injury.

Fig. 8. Mouse IFN-γ concentration in the serum was measured by ELISA using an anti-mouse IFN-γ mAb. The IFN-γ concentration was significantly decreased by HGF gene transfer or FK506 administration. Data represent means ± SD. *P < 0.01

Fig. 9. Immunohistochemistry (enzyme antibody staining). Tumors were removed at 14 days after BMT and investigated by immunohistochemical staining with the following antibodies: biotinylated H-2Kb (A), H-2Kk (B), CD4 (C), and CD8a (D) monoclonal antibody. H-2Kk and CD8 positive cells were detected in the tumors (arrows; original magnification ×20).
damage and subsequent inflammatory responses caused by acute GVHD of BMT recipients (13, 19). The intestinal damage caused by GVHD is initially induced by the apoptosis of epithelial cells (34). Subsequently, translocation of endotoxin from the gut into the portal circulation may lead to systemic GVHD (8). Since HGF has potent cytoprotective and antiapoptotic activity, HGF might reduce systemic GVHD by preventing gut injury. In this study, damage in the small intestines was observed in the GVHD control mice (group 3: TBI + BMT and group 4: TBI ± BMT with HVJ empty), such as atrophy of the villi and destruction of the crypts in the small intestine (Fig. 5, C and D) compared with group 1 mice (no treatment, Fig. 5A) or group 2 mice (TBI only, Fig. 5B). HGF gene transduction (group 5) or FK506 administration (group 6) significantly prevented these changes (Fig. 5, E and F). The average villus length of the GVHD control mice was significantly decreased compared with the HGF transduced mice or FK506 administered mice ($P < 0.01$, Fig. 7A). We also observed histological improvement in the large intestines by HGF or FK506 administration. Crypt destruction and loss of goblet cells were seen in the GVHD control mice, whereas these findings were clearly inhibited by HGF or FK506 (data not shown).

Figure 6 shows liver sections of each group (Fig. 6A: no treatment, Fig. 6B: TBI only). Hepatic GVHD is characterized by portal hepatitis, endothelialitis, and progression to nonsuppurative destructive cholangitis (31). Cellular infiltration in the periportal area is one of the representative findings of hepatic...

Fig. 10. Immunohistochemical analysis (double immunofluorescent staining). Tumors were removed at 14 days after BMT and investigated by double immunofluorescent staining using biotinylated anti-CD4, H-2K^k, and FITC-labeled anti-CD8, H-2K^k monochonal antibody: CD8 and CD4 (A), H-2K^k and H-2K^b (B), and CD8 and H-2K^k (C). Original magnification ×200. PE, phycoerythrin. CD8-positive lymphocytes were seen in the tumors from both the TBI-treated and TBI + BMT-treated mice (A). These lymphocytes were double positive for H-2K^b and H-2K^k in the tumors from mice not undergoing BMT, whereas the lymphocytes were H-2K^k positive but H-2K^b negative in the tumors from the TBI + BMT-treated mice (B). Merged image of CD8 and H-2K^k double staining indicates that these cells were H-2K^k/CD8 double positive (C).
GVHD. This finding was seen in the GVHD control mice (group 3; TBI ± BMT) and group 4 (TBI ± BMT with HVJ empty) (Fig. 6, C and D), but it was significantly inhibited by HGF transduction (group 5) or FK506 administration (group 6) (Fig. 6, E and F, and Fig. 7B; P < 0.01).

Effect of HGF on IFN-γ production during GVHD. IFN-γ primes macrophages, and these primed macrophages secrete inflammatory cytokines after stimulation by endotoxin during GVHD (23). This inflammatory cytokine cascade in the pathogenesis of both clinical and experimental GVHD is now well accepted (5, 9). The mean serum level of IFN-γ as follows: no treatment, 0.29 pg/ml; BMT, 618.2 pg/ml; BMT with HGF, 190.4 pg/ml; BMT with FK506, 105.6 pg/ml. The serum level of IFN-γ was significantly decreased by HGF treatment or FK506 administration (Fig. 8).

Donor-derived cytotoxic T cells killed tumor cells. To investigate whether donor CTLs (CD8^+ T cells) were the effector cells of the GVT effect, immunohistochemical analysis was performed. H-2K^k and CD8-positive cells were detected in the tumors of BMT recipients (Fig. 9, B and D). Double immunofluorescent staining was also examined by using biotinylated anti-CD4 mAb, anti-H-2K^k mAb, and FITC-labeled anti-CD8 mAb, anti-H-2K^b mAb. CD8 positive lymphocytes were seen in the tumors of both the BMT recipients and the mice receiving TBI (Fig. 10A). Tumor infiltrating lymphocytes were H-2K^b and H-2K^k positive in the mice receiving only TBI, indicating that these infiltrating lymphocytes were host origin. However, tumor infiltrating lymphocytes in the BMT recipients were H-2K^b negative and H-2K^k positive, indicating that these infiltrating lymphocytes were of donor origin (Fig. 10B). In addition, the merged images of CD8 and H-2K^k double staining indicates that the tumor-infiltrating cells in the BMT recipients were H-2K^b/CD8 double positive (Fig. 10C). These results indicate that donor-derived CD8 positive lymphocytes infiltrated into the tumors.

The cytotoxic activity of the BMT recipient’s spleen cells against Hepa1-a are shown in Fig. 10. EL4 was used as a target of donor anti-host CTL. YAC-1 was used as a target of NK cells. Spleen cells were harvested from the B6C3F1 recipients on day 10 after BMT, and the cytotoxic activities against Hepa1-a, EL4, and YAC-1 were tested after 3 days of in vitro sensitization. Spleen cells from the BMT recipients demonstrated strong cytotoxic activity against EL4 and Hepa1-a. In contrast, these cells did not exhibit cytotoxic activity against the YAC-1 cells (Fig. 11). These results suggest that donor-derived CTLs but not NK cells induced the GVT effect in this BMT model.

DISCUSSION

In this study, we evaluated the effect of allogeneic BMT with HGF gene transduction on HCC as a new strategy that could be an alternative to current treatments. Donor-derived CTLs killed HCC and markedly inhibited tumor growth after allogeneic BMT. Moreover, HGF gene transduction essentially suppressed GVHD, which is a serious complication of allogeneic BMT, whereas it preserved the GVT effects of BMT.

Immunotherapy for cancer is attractive because of the exquisite specificity of the immune response. Indeed, human clinical trials of immunotherapy for HCC were performed recently (2, 15, 16, 20, 27, 32, 36, 37). Among the various types of immunotherapy, allogeneic BMT is known to induce powerful GVT effects for patients with hematological malignancies and is currently well established as a treatment for some patients. This therapy also has been clinically used for some solid tumors and has demonstrated some clinical effects (3, 4, 40). The advantage of BMT is its systemic anti-tumor effects compared with other current therapies for HCC. Donor-derived T cells have the ability to kill tumors not only in the liver tumor lesion but also at distant metastatic sites. However, donor T cells not only contribute to the anti-tumor effects but also induce GVHD, which is a major complication of allogeneic BMT. Pharmacological immunosuppression is commonly used to control GVHD. However, immunosuppressive agents result in reduced GVT effects because they also inhibit donor T cell responses to host antigens. Indeed, FK506, a representative immunosuppressive agent, clearly inhibited GVHD but also reduced the GVT effects in this study.

Separation of the GVT effects from GVHD after BMT is thought to be crucial, and various animal studies have been reported: 1) inhibition of inflammatory cytokines using mAbs (10), 2) protection against intestinal damage using shielding cytokines (18), and 3) the administration of IL-12 or IL-18 (26, 44). Distinct from these strategies, we attempted to reduce GVHD by preventing intestinal injury using the biological effects of HGF.

HGF has mitogenic, motogenic, and morphogenic effects on various epithelial tissues. We previously demonstrated a beneficial effect of HGF gene transfer in mice with 2,4,6-trinitrobenzene sulfonic acid-induced colitis, which resembles human Crohn’s disease (24). HGF gene transfer significantly attenuates transmural inflammation by enhancing the repair of the epithelial lining. Since GVHD is initiated from intestinal injury and the subsequent entry of endotoxins from the gut lumen into the systemic circulation, we examined whether HGF can prevent GVHD by protection against intestinal injury. We demonstrated that HGF inhibited GVHD-mediated intestinal injury, thereby decreasing inflammatory cytokine production and donor T cell expansion (19). Moreover, we confirmed that HGF treatment preserved the GVT effects of BMT while reducing GVHD.

![Graph](http://ajpgi.physiology.org/)

**Fig. 11.** Cytotoxic activity of BMT recipient spleen cells. Effector cytotoxic T lymphocyte (CTL) activity was tested by the ^51^Cr release assay. Spleen cells freshly harvested from the BMT recipients were cultured with irradiated (20 Gy) B6C3F1 spleen cells in vitro. After 3 days of culture, the cells were harvested and CTL activity was examined on the basis of the lysis of Hepa1-a (H-2b, ●) or EL4 lymphoma cells (H-2b □) derived from C57BL/6 mice. To assess the NK cell activity, YAC-1 cells (▲) that are sensitive to natural killer cells were also used as a target. E/T ratio, effector-to-target cell ratio.
HGF had no effect on donor T cell proliferation in response to host antigens in vivo (13). Thus we utilized HGF to reduce GVHD; the influence of HGF on tumor proliferation should be considered because the effect of HGF on the growth of HCC is still controversial. Although tumorigenicity has been reported in transgenic mice overexpressing HGF, its HGF expression level was 5,000% higher in these mice compared with normal mice (35). On the other hand, Shiota et al. (30) reported that growth of HCC cell lines was inhibited by the addition of recombinant HGF (50–200 ng/ml). Moreover, transgenic mice that express HGF 200–300% higher than that of normal mice inhibit the development of neoplastic tumors (28). We assessed the effect of HGF on Hepa1-α cell proliferation in vitro with 1–20 ng/ml of recombinant HGF because HGF gene transduced mouse serum HGF level was less than 2 ng/ml. No significant effect was seen on cell proliferation such a low HGF levels. Therefore, these results suggested that suppression of tumor growth was mediated by BMT, not HGF.

We used HVJ-liposome as HGF gene expression system in our present study. To apply BMT/HGF strategy to human clinical use, we do not stick to gene transduction of HGF. We consider that most required factors for clinical use are safe for humans and that the HGF level is stable in vivo. In our previous study, a high HGF expression level, such as mediated adenoviral vector, is not necessarily required for various HGF treatments (24, 39). From this point of view, recombinant HGF administration is also suitable candidate for clinical use.

In conclusion, we demonstrated that allogeneic BMT markedly suppressed the growth of HCC. HGF gene transfer simultaneously suppressed GVHD by decreasing intestinal injury and the subsequent entry of endotoxins from the gut lumen into the systemic circulation while preserving the GVT effect. Various therapeutic strategies for separation of the GVT effect from the occurrence of GVHD after BMT have been examined in animal models. However, this is the first report to demonstrate separation of the GVT effect from GVHD in a solid tumor.

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