Transfection with a bcl-2 Expression Vector Protects Transplanted Bone Marrow from Chemotherapy-induced Myelosuppression

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ABSTRACT

The use of cytokines such as granulocyte-colony-stimulating factor (G-CSF) to ameliorate chemotherapy-induced myelosuppression may not only stimulate the recovery of normal hematopoietic cells but may also enhance the proliferation of the tumor cells with functional receptors for these cytokines. In this study, we show that administration of recombinant human (rh) G-CSF decreased the in vitro and in vivo cytotoxic effects of Adriamycin or etoposide on L1210 murine leukemia cells with receptors for rhG-CSF. Transplantation of bone marrow cells expressing high levels of bcl-2 from a retroviral construct [MPZenNeo(bcl-2)] (bcl-2-BMT) did not decrease the in vivo cytotoxic effect of etoposide on L1210 cells, but enabled recovery of myelopoiesis following etoposide-induced myelosuppression to almost the same extent as did the administration of rhG-CSF. These findings suggest the possibility that bcl-2 transfection could be used to protect transplanted bone marrow from chemotherapy-induced myelosuppression on behalf of administration of rhG-CSF, in case of treatment of tumors with functional receptors for rhG-CSF.

INTRODUCTION

An increase in the dose of chemotherapy enhances the response of many experimental and clinical tumors, but the extent of dose escalation is often limited by myelosuppression. Therefore, reduction in the degree or duration of chemotherapy-induced myelosuppression may decrease the morbidity of chemotherapy and enhance tumor control.

The recent molecular cloning and expression of four human CSFs [G-CSF, GM-CSF, M-CSF, and interleukin 3] have made it possible to accelerate hematopoietic recovery from myelosuppression (1-4). However, these CSFs have been shown to modulate the growth of leukemic cells in vitro (5-10). More recently, Lotem and Sachs (11) have suggested that the use of cytokines such as G-CSF or GM-CSF to ameliorate myelosuppression that follows the use of chemotherapeutic agents or radiation in cancer therapy may not only stimulate the recovery of normal hematopoietic cells but may also enhance the proliferation of the leukemic cells or nonhematopoietic tumor cells with functional receptors for these cytokines. Therefore, in case of treatment of malignant diseases with functional receptors for G-CSF or GM-CSF, the application of novel treatments other than the administration of these cytokines may be necessary for amelioration of the myelosuppression induced by chemotherapy.

In this study, we first show that administration of rhG-CSF decreases the in vitro and in vivo cytotoxic effects of Adriamycin or VP-16 on L1210 murine leukemic cells with receptors for rhG-CSF. Furthermore, we have recently obtained evidence that the enforced expression of bcl-2 gene in murine BM cells enables rescue from in vitro myelosuppression induced by topoisomerase I or II inhibitors such as camptothecin, Adriamycin, and VP-16. Therefore, we wished to determine whether the bcl-2 gene enables rescue from in vivo myelosuppression induced by chemotherapy without inhibiting the antitumor activity in L1210 cells by chemotherapeutic agents. Then, we introduced a human bcl-2 gene into BM cells obtained from BALB/c × DBA/2 F1 (hereafter called CD2F1) mice by MPZenNeo(bcl-2) retroviral gene transfer (12, 13), and bcl-2-expressing BM cells were injected i.v. into lethally irradiated syngeneic mice. In this study, we also show that bcl-2-BMT does not decrease the in vivo cytotoxic effect of VP-16 on L1210 cells, but enables recovery of myelopoiesis following VP-16-induced myelosuppression to almost the same extent as does the administration of rhG-CSF.

MATERIALS AND METHODS

Reagents. rhG-CSF was kindly supplied by CHUGAI Pharmaceutical Co., Ltd. (Tokyo, Japan). Adriamycin was kindly supplied by Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan). These agents were obtained in powder form, from which 250 µg/ml or 1.0 mg/ml stock solution was prepared in normal saline, respectively. VP-16 was kindly supplied by Nippon Kayaku Co. (Tokyo, Japan). It was also obtained in powder form, from which 10 mg/ml stock solution was prepared in dimethyl sulfoxide.

Tumor Cells. L1210 murine leukemia cells were kindly provided by the Japanese Cancer Research Resources Bank (Tokyo, Japan). L1210 cells were maintained in suspension culture in Fischer's medium supplemented with 4 mM glutamine, 10% FCS (GIBCO, Grand Island, NY), and the antibiotics penicillin and streptomycin. To determine whether L1210 cells express receptors for rhG-CSF, binding assay of 125I-rhG-CSF to L1210 cells was performed as previously described (14). rhG-CSF was radiiodinated with Na125I by the two-phase chloramin T method. This procedure yielded a high specific radioactivity (about 5 × 107 cpm/µg).

Effect of rhG-CSF on the in Vitro Antitumor Activity by Adriamycin or VP-16. The effect of rhG-CSF on cytotoxicity in L1210 cells induced by Adriamycin or VP-16 was quantified by using a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide (CHEMICON, Temecula, CA) colorimetric assay (15). Tumor cells were seeded at 104 cells/well (0.1 ml) in 96-well flat-bottomed plates (Corning, NY) and treated with varying doses of these chemotherapeutic agents without or with 10.0 ng/ml rhG-CSF. After a 48-h period of incubation at 37°C, 0.01 ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reagent was added to each well. After another 4-h period of incubation at 37°C, 0.1 ml of isopropyl alcohol with 0.04 N HCl was added to each well to dissolve precipitates, and absorbance was then measured at 570 nm with an autoreader (ER-8000, Sanko Junyaku Co., Ltd., Tokyo, Japan) within 30 min of dissolution. Student's t test was used to assess the statistical significance of the observed differences.

Analysis of DNA Fragmentation in Agarose Gel. This assay was performed as previously described (16). Briefly, harvested L1210 cells (1 × 107) were centrifuged and washed twice with cold PBS. The cell pellet was lysed in 1.0 ml of a buffer consisting of 10 mM Tris-HCl, 10 mM EDTA, and 0.2% Triton X-100 (pH 7.5). After 10 min on ice, the lysate was centrifuged (13,000 × g) for 10 min at 4°C in an Eppendorf microtube. Then, the supernatant...
(containing RNA and fragmented DNA, but not intact chromatin) was extracted first with phenol and then with phenol-chloroform-isooamyl alcohol (24:1). The aqueous phase was made up to 300 mM NaCl and nucleic acids were precipitated with 2 volumes of ethanol. The pellet was rinsed with 70% ethanol, air dried, and dissolved in 20 μl of 10 mM Tris-HCl-1 mM EDTA (pH 7.5). Following digestion of RNA with RNase A (0.6 mg/ml, at 37°C for 30 min), the sample was electrophoresed in a 2% agarose gel with Boyer’s buffer (50 mM Tris-HCl, 20 mM sodium acetate, 2 mM EDTA, and 18 mM NaCl, pH 8.05). DNA was then visualized with ethidium bromide staining.

BM Cultures and BMT. BM cells were obtained from the femurs of 3-week-old CD2F1, mice. Marrow plugs were flushed out with PBS, passed through a 27-gauge needle, washed, and resuspended in RPMI 1640 medium supplemented with 10% FCS, the antibiotics penicillin and streptomycin, and 5 × 10−5 M 2-mercaptoethanol at a density of 10⁶ cells/ml. Macrophages and other adherent cells were removed after incubation for 18 h at 37°C in 5% CO₂ in tissue culture flasks as described previously (17). Nonadherent cells were collected and resuspended in complete RPMI medium and cultured with 10.0 ng/ml rhG-CSF. Nonadherent BM cells (5 × 10⁶/0.2 ml PBS) were injected i.v. into lethally irradiated (9.5 Gy) syngeneic CD2F1, mice. These mice were given i.p. 5 μg/kg rhG-CSF daily for 1 week, designated as BMT mice at 14 days posttransplantation, and were used as in vivo experimental models.

bcl-2 Transfection in BM Cells and Immunoprecipitation. A 942-base pair blunt-end EcoRI/TaqI complementary DNA fragment containing the entire human bcl-2 coding sequence (13) was inserted into the blunt-ended XhoI site of the retroviral vector MIPZenSVNeo, allowing expression of bcl-2 from MPSV long terminal repeat (12, 18). Murine fibroblast lines secreting MIPZenNeo (bcl-2) or MIPZenNeo virus free of helper virus were obtained by electroporating the M2 packaging line (19) with retroviral plasmid DNA. Nonadherent BM cells were infected by adding the filtered supernatant of the virus-producing 62 fibroblast cells having a titer of 1.0 × 10⁶/ml (20) with incubation for 24 h. No growth factor was used to stimulate gene transfer. Infected BM cells were selected in 800 μg/ml G 418. 0 418-resistant cells were determined by an indirect immunofluorescence method by using anti-human bcl-2 MoAb (Biochemicals, Tokyo, Japan) (positive cells >90%, data not shown) (21). In addition, the production of the bcl-2 gene in BM cells was determined by an indirect immunofluorescence method by using anti-human bcl-2 MoAb. Briefly, BM cells were washed with methionine-free medium containing 5% FCS, suspended at 2.5 × 10⁶ cells/ml in the same medium supplemented with 250 μCi/ml L-[35S]methionine (Amersham, Arlington Heights, IL) and cultured for 5 h. The cells were harvested, washed with PBS, and lysed as previously described (21). The lysate was mixed with anti-human bcl-2 MoAb, and the immune complexes were precipitated with protein A-Sepharose (Amersham) and analyzed on a 12% sodium dodecyl sulfate-polyacrylamide gel.

Effect of bcl-2-BMT and rhG-CSF on the In Vivo Antitumor Activity by VP-16. Non-BMT, parental-BMT, or bcl-2-BMT mice were inoculated i.p. with L1210 cells (1 × 10⁶ cells/animal) on Day 0 (at 14 days posttransplantation) and were given i.p. 32.0 mg/kg VP-16 on Days 1 to 3 daily. Daily i.p. administration of 5 μg/kg rhG-CSF was carried out from Day 1 to Day 7. The relative survival rate for mice was calculated as follows.

\[
\text{T/C} = \frac{\text{Mean survival days of VP-16- or rhG-CSF-treated mice}}{\text{Mean survival days of untreated mice}} \times 100
\]

Student’s t test was used to assess the statistical significance of the observed differences.

Effect of bcl-2-BMT and rhG-CSF on Myelosuppression Induced by VP-16. To determine morphologically whether apoptosis is induced in BM cells by VP-16, nonadherent BM cells obtained from BMT mice 7 days after treatment with VP-16 were examined at the ultrastructural level. Briefly, 2 × 10⁶ BM cells were harvested, washed in PBS, pelleted, prefixed in 2.0% glutaraldehyde for 2 h, and washed in 0.1 M phosphate buffer (pH 7.4), followed by postfixation with 1.0% osmium tetroxide for 2 h. Samples were embedded in Epon 812, sectioned, and stained for 20 min in 2.0% aqueous uranyl acetate and for 2 min in lead citrate. Grids were viewed with the use of a JEM-1200EX electron microscope (NEC, Tokyo, Japan). In addition, VP-16-treated or untreated BM cells were bled retroorbitally for determination of neutrophil counts. Moreover, in order to evaluate the proliferation activity of BM cells from BMT mice, granulocyte colony formation by BM cells of each

![Fig. 1. Inhibition of Adriamycin- or VP-16-induced apoptosis by rhG-CSF. Degree of inhibition of Adriamycin- or VP-16-induced cytotoxicity in L1210 cells by rhG-CSF, as determined by using a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. rhG-CSF (10 ng/ml) inhibited the loss of L1210 cell viability induced by Adriamycin (A, D) or VP-16 (B, 0) (P < 0.05 or P < 0.1, respectively). Points, mean value from three separate experiments. Induction of DNA fragmentation was assessed for L1210 cells treated with Adriamycin (10 μg/ml) or VP-16 (10 μg/ml) without or with rhG-CSF (10 ng/ml) for 48 h (C). Fragmented DNA was electrophoresed in a 2.0% agarose gel containing 0.5 μg/ml ethidium bromide. Lanes 2 and 4. L1210 cells treated with Adriamycin alone or VP-16 alone, respectively. Lanes 3 and 5. L1210 cells treated with both Adriamycin and rhG-CSF or both VP-16 and rhG-CSF, respectively. Molecular weight standards of multiples of 123-base pair DNA ladder (GIBCO BRL, Tokyo) are shown in Lane 1.](image-url)
RhG-CSF Inhibited the in Vitro Cytotoxic Effect of Adriamycin or VP-16. As shown in Fig. 1, A and B, rhG-CSF significantly inhibited the loss of L1210 cell viability induced by Adriamycin or VP-16 (P < 0.05 or P < 0.1, respectively). In addition, rhG-CSF almost inhibited DNA fragmentation induced by Adriamycin or VP-16 in L1210 cells (Fig. 1C). These results show that administration of rhG-CSF inhibited the in vitro antitumor activity and induction of apoptosis in L1210 cells by Adriamycin or VP-16. On the other hand, the administration of rhG-CSF enhanced the in vitro growth of L1210 cells (data not shown). Moreover, \(^{125}\)I-rhG-CSF was found to bind specifically to L1210 cells (Fig. 2). The specific binding was saturable, whereas nonspecific binding increased linearly with increasing concentrations of labeled rhG-CSF (Fig. 2A). Scatchard plot of the binding data revealed that L1210 cells expressed 398 receptors per cell (Fig. 2B).

Expression of bcl-2 Gene in BM Cells. Expression of the introduced bcl-2 gene in BM cells was confirmed by immunoprecipitation with the use of anti-human bcl-2 MoAb (Fig. 3). In addition, bcl-2 expression of nonadherent BM cells obtained from mice 4 weeks after bcl-2-BMT appeared to be slightly decreased but was maintained during at least this period.

Effect of bcl-2-BMT and rhG-CSF on the in Vivo Antitumor Activity by VP-16. As shown in Table 1, the treatment of rhG-CSF alone in non-BMT, parental-BMT, or bcl-2-BMT mice promoted the growth of L1210 cells since the survival time was shortened significantly compared to no treatment group (P < 0.1, P < 0.1, or P < 0.05, respectively). As in vitro study predicted, the combined therapy with VP-16 and rhG-CSF in non-BMT, parental-BMT, or bcl-2-BMT mice significantly decreased the cytotoxic effect of VP-16 alone on L1210 cells (P < 0.1, P < 0.05, or P < 0.1, respectively). In addition, the treatment of VP-16 or rhG-CSF did not induce any difference among non-BMT, parental-BMT, and bcl-2-BMT groups. These results show that rhG-CSF enhanced the in vivo proliferation of L1210 cells and decreased the in vivo effect of VP-16 on L1210 cells, and that enforced expression of bcl-2 in BM cells did not decrease the effect of VP-16 or rhG-CSF on L1210 cells.

Effect of bcl-2-BMT and rhG-CSF on Myelosuppression Induced by VP-16. Fig. 4 shows that nonadherent BM cells obtained on Day 7 from mice treated with VP-16 after parental-BMT lost viability, and frequently displayed typical apoptotic morphology including chromatin condensation, while the viability of BM cells from bcl-2-BMT appeared to be slightly decreased but was maintained during at least this period.

RESLISTS

\[ \text{Effect of bcl-2-BMT and rhG-CSF on the in vivo antitumor activity in L1210 cells by VP-16} \]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival time (days, mean ± SD)</th>
<th>T/C (%)</th>
<th>Survivors on Day 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-BMT group (control)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td>10.0 ± 0.5</td>
<td>100</td>
<td>0/5</td>
</tr>
<tr>
<td>rhG-CSF (^{b})</td>
<td>7.6 ± 0.4(^{c})</td>
<td>76</td>
<td>0/5</td>
</tr>
<tr>
<td>VP-16</td>
<td>18.4 ± 1.7</td>
<td>73</td>
<td>0/5</td>
</tr>
<tr>
<td>VP-16 + rhG-CSF</td>
<td>13.3 ± 0.7(^{e})</td>
<td>133</td>
<td>0/5</td>
</tr>
<tr>
<td>Parental-BMT group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td>9.8 ± 0.4</td>
<td>100</td>
<td>0/5</td>
</tr>
<tr>
<td>rhG-CSF</td>
<td>7.2 ± 0.5(^{c})</td>
<td>73</td>
<td>0/5</td>
</tr>
<tr>
<td>VP-16</td>
<td>19.2 ± 1.4</td>
<td>196</td>
<td>0/5</td>
</tr>
<tr>
<td>VP-16 + rhG-CSF</td>
<td>14.6 ± 1.2(^{c})</td>
<td>122</td>
<td>0/5</td>
</tr>
<tr>
<td>bcl-2-BMT group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td>10.3 ± 0.5</td>
<td>100</td>
<td>0/5</td>
</tr>
<tr>
<td>rhG-CSF</td>
<td>6.9 ± 0.4(^{c})</td>
<td>67</td>
<td>0/5</td>
</tr>
<tr>
<td>VP-16</td>
<td>18.7 ± 1.5</td>
<td>182</td>
<td>0/5</td>
</tr>
<tr>
<td>VP-16 + rhG-CSF</td>
<td>14.1 ± 0.9(^{c})</td>
<td>137</td>
<td>0/5</td>
</tr>
</tbody>
</table>

Five CD2F1 mice in each treatment were examined.

\[ T/C(%) = \frac{\text{Mean survival days of VP-16- or rhG-CSF treated mice}}{\text{Mean survival days of untreated mice}} \times 100 \]

\(^{b}\) 5 μg/kg, i.p., Days 1–7.

\(^{c}\) P < 0.1 compared with corresponding no treatment group.

\(^{d}\) 320 μg/kg, i.p., Days 1–3.

\(^{e}\) P < 0.1 compared with corresponding VP-16-treated group.

\(^{f}\) P < 0.05 compared with corresponding VP-16 treated group.

\(^{g}\) P < 0.05 compared with corresponding no treatment group.

\[ \text{Fig. 2. Binding of radiolabeled rhG-CSF to L1210 cells. L1210 cells (4 × 10^4) were incubated with increasing amounts of radiolabeled rhG-CSF for 1 h at 37°C. Nonspecific binding was determined in the presence of excess unlabeled rhG-CSF. Specific binding (\(\triangle\)) and nonspecific binding (\(\Box\)) data are plotted (A). (B) indicates Scatchard plot of these data.} \]
bcl-2 PROTECTS BM TRANSPLANT FROM MYELOSUPPRESSION

with functional receptors for these cytokines (5—11). In this study, and in agreement with these previous reports, we first demonstrated that rhG-CSF inhibited the in vitro and in vivo antitumor activity and induction of apoptosis in L1210 cells caused by treatment with Adriamycin or VP-16, and that L1210 cells had receptors for rhG-CSF. Therefore, we wished to examine whether there was any novel treatment other than the administration of rhG-CSF which ameliorated myelosuppression but did not decrease the cytotoxic effect of chemotherapeutic agents on L1210 cells.

Recently, camptothecin and VP-16 have been shown to induce apoptosis with DNA cleavage in thymocytes (29, 30) and in concanavalin A-stimulated splenocytes (31). In addition, Adriamycin is known to induce apoptosis in the murine small intestinal tract (32) and in thymocytes (30). More recently, we have found that topoisomerase inhibitors induce apoptosis in murine BM cells in vitro. Moreover, our present results clearly indicate that VP-16 induces apoptosis in BM cells even in an in vivo model. Taken together, we suggest the hypothesis that the main mechanism of myelosuppression may be the apoptosis in BM cells induced by chemotherapeutic agents. In this study, therefore, we wished to determine whether bcl-2 enables recovery of myelopoiesis following chemotherapy-induced myelosup-

mice treated with VP-16 after bcl-2-BMT remained high. In addition, BM cells from mice treated with both VP-16 and rhG-CSF after parental-BMT showed almost normal viability (data not shown). As shown in Fig. 5, rhG-CSF accelerated neutrophil recovery in VP-16-treated mice after parental-BMT or bcl-2-BMT, with normal levels achieved in 8 or 7 days in contrast to about 12 days required in control group, respectively. In addition, the treatment with VP-16 decreased neutrophil numbers in the bcl-2-BMT group. However, at no stage in this group were neutrophils absent from the circulation. On the other hand, there was no difference in rhG-CSF-induced granulocyte colony formation of BM cells obtained from non-BMT, parental-BMT, and bcl-2-BMT mice at 1 week posttreatment of rhG-CSF (Fig. 6). Therefore, we suggest that BM cells of parental-BMT and bcl-2-BMT mice possess the proliferation activity to the same extent as do those of non-BMT mice. These results show that bcl-2-BMT enabled recovery of myelopoiesis following VP-16-induced myelosuppression to almost the same extent as did administration of rhG-CSF.

DISCUSSION

Drugs that interact with DNA topoisomerase, such as camptothecin, Adriamycin, and VP-16, have been found to be particularly useful in chemotherapeutic treatment, but tend to induce myelosuppression (23—25). In order to ameliorate myelosuppression induced by these chemotherapeutic agents, rhG-CSF or rhGM-CSF have been used at present (26—28). However, these CSFs have been shown to not only ameliorate myelosuppression but also modulate the growth of tumors

Fig. 4. Ultrastructural appearance of BM cells obtained on Day 7 from mice treated with VP-16 (32.0 mg/kg, i.p., Days 1—3) after parental-BMT (A) or bcl-2-BMT, (B) (B) (× 7200). Arrow indicates condensed chromatin.

Fig. 5. Neutrophil counts in mice treated with VP-16 (32.0 mg/kg, i.p., Days 1—3) and rhG-CSF (5 μg/kg, i.p., Days 1—7). U, control (non-BMT) mice treated with VP-16 alone; ▲, parental-BMT mice treated with VP-16 alone; ●, parental-BMT mice treated with VP-16 and rhG-CSF; ◇, bcl-2-BMT mice treated with VP-16 alone; and ○, bcl-2-BMT mice treated with VP-16 and rhG-CSF. Non-BMT and parental-BMT mice treated with VP-16 alone had no detectable neutrophils between Days 5 and 8 or Days 5 and 7, respectively. Results are for groups of five CD2F1 mice. Points, mean; bars, SE.

Fig. 6. Granulocyte colony formation of BM cells by rhG-CSF; 2-fold diluted rhG-CSF was titrated by colony-forming assay in soft agar. ●, control (BM cells from non-BMT mice); △, BM cells from parental-BMT mice; and ○, BM cells from bcl-2-BMT mice.

DISCUSSION

Drugs that interact with DNA topoisomerase, such as camptothecin, Adriamycin, and VP-16, have been found to be particularly useful in chemotherapeutic treatment, but tend to induce myelosuppression (23—25). In order to ameliorate myelosuppression induced by these chemotherapeutic agents, rhG-CSF or rhGM-CSF have been used at present (26—28). However, these CSFs have been shown to not only ameliorate myelosuppression but also modulate the growth of tumors

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pression. Then, we introduced a human bcl-2 gene into murine BM cells by retroviral gene transfer, and consequently demonstrated that transplantation of BM cells expressing high levels of bcl-2 ameliorated VP-16-induced myelosuppression, but did not decrease the in vivo cytotoxic effect of VP-16 on L1210 cells.

The protooncogene bcl-2 was discovered as a result of its translocation to the immunoglobulin heavy chain locus in most cases of human follicular center B-cell lymphoma (12, 20, 33). This t(14;18) chromosomal translocation spares the coding region of the bcl-2 gene but appears to deregulate its expression. The bcl-2 gene encodes a cytoplasmic protein (34, 35) that appears to be associated with the inner and outer membranes of the mitochondria, nuclear envelope, and endoplasmic reticulum (36–38). Insight into the recent biological function of bcl-2 came with the discovery that enforced bcl-2 expression delays the death of certain hematopoietic cell lines deprived of growth factor (12). Moreover, transgenic mice expressing a bcl-2 gene subjected to an immunoglobulin enhancer contain a large excess of B-lymphocytes with enhanced survival capacity, which may progress into high grade lymphoma or autoimmune disease (39–41). Taken together, it will be a problem to introduce bcl-2, an oncogene, into human BM, as it could cause tumors or autoimmune disease. More recently, bcl-2 is shown to be a homologue of the Caenorhabditis elegans gene ced-9 (42), which inhibits the activity of ced-3. The amino acid sequence of Ced-3 protein is similar to the mammalian interleukin 1β-converting enzyme (43, 44). Therefore, inhibition of interleukin 1β-converting enzyme expression may protect BM from chemotherapy-induced myelosuppression.

Recently, Miyashita et al. have demonstrated that bcl-2 gene transfer increases relative resistance of murine lymphoid cells and human leukemia cells to cell death and DNA fragmentation induced by chemotherapeutic drugs such as methotrexate, vincristine (45), and VP-16 (46). In addition, we have recently found that bcl-2 prevents apoptosis of pituitary adenoma cells induced by bromocriptine. The bcl-2 gene might therefore interfere with a final common pathway for cell death that can be activated by multiple mechanisms.

In conclusion, our findings suggest the possibility that bcl-2 transfection could be used to protect transplanted BM from chemotherapy-induced myelosuppression on behalf of administration of rhG-CSF, in case of treatment of tumors with functional receptors for rhG-CSF. We are at present attempting to determine whether bcl-2 transfection in BM cells remains to be persistent, prevents apoptosis in thymus, induces tumors/autoimmune disease, or induces the production of murine antibody against human bcl-2 protein, which may induce graft rejection by antigen-antibody reaction.

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