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Treatment of Murine Tumors with Lethal Doses of Dimethylmyleran and Autologous Bone Marrow*

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Abstract—*A murine model for autologous bone marrow transplantation is presented. Up to 90% of CBA and Balb/c mice survived a lethal dose of dimethylmyleran (DMM) if they were infused intravenously with autologous marrow cells removed prior to the injection of the drug. Autologous or syngeneic hemopoietic cells afforded equivalent protection. A significant proportion of CBA mice carrying the syngeneic YBA lymphoma and of Balb/c mice inoculated with the syngeneic Meth A sarcoma enjoyed lasting tumor-free remissions after treatment with a single lethal dose of DMM and autologous marrow. Sublethal doses of DMM failed to eradicate the tumors. Although curative superdosis tumor chemotherapy with autologous marrow was demonstrated, potential difficulties consist in the increased susceptibility of tumor bearing mice to the cytotoxic drug and in the contamination of the marrow as early as one day after intraperitoneal tumor inoculation.*

INTRODUCTION

IN SPITE of progress with allogeneic human marrow transplantation after total body irradiation (TBI) in the treatment of acute leukemia, problems such as relapse, rejection of allogeneic marrow and fatalities due to graft-versus-host disease remain prominent [1, 2]. A protocol of high-dose chemotherapy and autologous hemopoietic reconstitution is likely to offer advantages since leukemic relapse may be reduced with the substitution of TBI by cytotoxic drugs and graft rejection and graft-versus-host disease are avoided with autologous marrow. This approach might be feasible [3–5] in malignancies without prohibitive bone marrow involvement.

DMM is a cytotoxic agent which seems particularly suitable for superdosis tumor chemotherapy. As it displays an unusually selective hemopoietic toxicity, its lethal effects can be reversed in animals [6–8] and humans [9] by syngeneic marrow infusion. Also, DMM does not depress markedly the immune response [10, 11]. This may be a relevant advantage in cancer chemotherapy since malignant cells are more vulnerable to a combined pharmacological and immunological cell destruction [12–14]. The mode of action of DMM, affecting like X-rays both resting and proliferating cells [15, 16] makes it an appropriate agent for single supralethal doses followed by bone marrow.

In earlier studies, syngeneic murine lymphomas or leukemias were successfully treated with lethal doses of DMM followed by syngeneic bone marrow [14] or immune spleen cells [17]. The present experiments were performed to provide an experimental model for clinical studies involving superdosis chemotherapy with DMM and autologous marrow rescue.

MATERIALS AND METHODS

Mice

Adult female CBA or Balb/c mice (Gl. Bomholtgard, 8680 Ry, Denmark) weighing 18–25 g were used. They received NAFAG pellets and sterile water *ad libitum*.

Tumors

The syngeneic YBA Moloney lymphoma was obtained from Prof. George Klein (Karolinska Institute, Stockholm) and the methylcholantrene-induced sarcoma (Meth A) from Dr. A. Matter (F. Hoffmann–La Roche & Co AG, Basle). The tumors were maintained in female CBA or Balb/c mice by subcutaneous or in-

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traperitoneal inoculation. Graded tumor cell doses suspended in tissue culture medium TC 199 were made up for the experiments and injected in aliquots of 0.05 ml (subcutaneously) or 0.5 ml (intraperitoneally) per mouse. Survival was followed.

Preparation of bone marrow and spleen cells

Syngeneic cells were prepared as described previously [14]. Autologous bone marrow cells were obtained by amputation of one hind leg. For this purpose, intraperitoneal nembutal anesthesia supplemented locally with 1% lidocaine was applied. The leg was disarticulated at the ilio-femoral joint, blood vessels were ligated or cauterized and skin closure performed with Michel clamps. The amputation was supported well and did not affect the mobility of the mice.

Splenectomies were performed also in nembutal anesthesia by ligature of the spleen stem, removal of the organ and closure of the abdominal wall and skin by Michel clamps. About 15×10^6 (Balb/c mice) or 25×10^6 (CBA mice) bone marrow or 100×10^6 spleen cells were harvested from each mouse. The cells from each mouse were stored individually in 15 ml plastic tubes (Corning) in TC 199 with 2% fetal calf serum (FCS) at 4°C. Viability after overnight storage was estimated with the trypan blue method and amounted to 80–90%. All cell injections were performed intravenously after centrifugation and resuspension to the desired volume in 0.5 ml of TC 199. The respective controls received only TC 199.

Drugs

DMM (1,4-dimethanesulfonoxy-1,4-dimethylbutane) was kindly provided by Dr. Harry B. Wood, Division of Cancer Treatment, NCI, National Institute of Health, Bethesda, Maryland (USA). It was first dissolved in 94% ethanol, to which warm sterile saline was added to make up for applying the desired dose intraperitoneally in 0.2 ml per 10 g body weight.

Experimental protocol

DMM was injected 5–6 hr after the removal of hemopoietic cells. The harvested cells from each mouse were stored individually and reinjected to the respective donors within 15–20 hr after the DMM. Syngeneic bone marrow cells were obtained from 8 to 12 weeks old female CBA donors and administered after having been stored similarly. In the experiments involving superdosis tumor chemotherapy, autologous cells were removed at the indicated intervals (Table 2) after the tumor challenge and reinjected as described.

Contamination of autologous marrow with tumor cells

Female CBA mice (primary recipients) were injected by various routes with 10^6 YBA cells. Three days later, 10×10^6 bone marrow cells of these animals were injected intravenously into normal CBA females (secondary recipients) whose survival was recorded. Also, one day after the inoculation of primary recipients with 10^6 YBA cells, 10×10^6 bone marrow cells were removed and injected intravenously into secondary recipients 6 hr after the administration of 18.5 mg/kg DMM by the intraperitoneal route. The survival of these secondary recipients was followed.

Statistical analysis

The significance of the results was determined by the chi-square test.

RESULTS

Therapy of YBA lymphoma with DMM and syngeneis restoration

As shown in Table 1, the survival of mice challenged subcutaneously with 10^6 Moloney lymphoma cells was increased by treatment with 18.5 mg/kg DMM and syngeneic bone marrow from 0 to 50% ($P < 0.005$). With intraperitoneal tumor inoculation, survival was increased to 83% ($P < 0.0005$). Therapy with 15 mg/kg DMM and syngeneic bone marrow did not allow significant survival. DMM (18.5 mg/kg) without subsequent syngeneic marrow was fatal to all mice (groups 2 and 5) while only 3 out of 18 mice treated with DMM and marrow succumbed to DMM toxicity (groups 3 and 6). Eleven (73%) of the 15 mice surviving the treatment with DMM and syngeneic marrow remained tumor-free while 4 (27%) died with tumors.

Therapy of YBA lymphoma with DMM and autologous restoration

Essentially similar results were obtained if autologous bone marrow or spleen cells were used to restore hemopoiesis after a lethal dose of DMM (Table 2). Group 1 demonstrates that 89% of the mice survived 18.5 mg/kg DMM if autologous bone marrow cells were reinfused. If this protocol was applied to tumor-bearing mice, survival was increased from 12 to 70% ($P < 0.0005$) after a subcutaneous challenge with 10^4 tumor cells (groups 2 + 3 vs 4) and from 0 to 100% ($P < 0.0005$) with a challenge of 10^5 tumour cells (groups 5 + 6 vs 7). A challenge of 5×10^5 tumour cells was survived by only 1 out of 11 mice (combined groups 8 and 10), but by 8 out of 11 mice (combined groups 9 and 11;

Table 1. *Effect of DMM combined with syngeneic bone marrow cells (SBM) on the survival of CBA mice inoculated with* 10^6 *Moloney lymphoma cells*

Group No.	Route of tumor inoculation	DMM (mg/kg)	SBM ($\times 10^6$)	No. of mice per group	No. dying without tumors before D20	No. dying with large tumors after D30	No. (and %) surviving without tumor
1	s.c.	—	—	12	0	12	0
2	s.c.	18.5	—	12	12	0	0
3	s.c.	18.5	25	12	2	4	6 (50)
4	i.p.	—	—	12	0	12	0
5	i.p.	18.5	—	6	6	0	0
6	i.p.	18.5	25	6	1	0	5 (83)
7	i.p.	15	—	6	4	1	1 (17)
8	i.p.	15	25	6	1	5	0

Panels of female CBA mice were inoculated subcutaneously (s.c.) or intraperitoneally (i.p.) with 10^6 Moloney Lymphoma cells on day (D) 0. On Dl, 18.5 or 15 mg/kg DMM were injected intraperitoneally followed (group 3, 6 and 8) on D2 by an intravenous injection of 25×10^6 syngeneic bone marrow cells. Fatalities due to DMM toxicity occured in mice without evidence of tumor before D20. Death due to tumor development with large subcutaneous masses or ascites supervened between D30 and 50. Since no tumors were seen arising after day 50, the experiment was terminated on D60 and the tumor-free mice were considered as survivors. The statistical significance of the differences in the survival rates (chi-square test) were as follows: group 3 vs 1: $P < 0.005$; group 6 vs 4: $P < 0.0005$.

Table 2. Survival of female CBA mice challenged subcutaneously with various doses of Moloney lymphoma cells and treated with 18.5 mg/kg DMM and autologous bone marrow or spleen cells

Group No.	No. of tumor cells	Amputation on D	DMM injected on D	Reinjection of autologous cells on D	No. of mice per group	No. dying without tumors before D20	No. dying with large tumors after D30	No. (and %) surviving without tumor
1	—	1	1	2	37	4	0	33 (89)
2	10^4	—	—	—	7	0	6	1 (17)
3	10^4	1	—	2	10	0	9	1 (10)
4	10^4	1	1	2	20	6	0	14 (70)
5	10^5	—	—	—	8	0	8	0
6	10^5	1	—	2	8	0	8	0
7	10^5	1	1	2	8	0	0	8 (100)
8	5×10^5	1	—	2	5	0	4	1 (20)
9	5×10^5	1	1	2	6	0	1	5 (83)
10	5×10^5	1*	—	2*	6	0	6	0
11	5×10^5	1*	1	2*	5	1	1	3 (60)
12	10^6	—	—	—	10	0	9	1 (10)
13	10^6	1	—	2	10	0	10	0
14	10^6	1	1	2	9	4	1	4 (44)
15	10^6	3	3	4	10	10	0	0
16	10^6	9	9	10	11	3	5	3 (27)

*In groups 10 and 11, mice were splenectomized and reinfused with autologous spleen cells.

The significances (chi-square test) of the differences in survival rates were as follows: group 4 vs 2+3: $P < 0.0005$; group 7 vs 5+6: $P < 0.0005$; groups 8+10 vs 9+11: $P < 0.005$; group 14 vs 12+13: $P < 0.01$ and group 16 vs 12+13: $P < 0.1$.

$P < 0.005$) if they were treated with a lethal dose of DMM followed by intravenous autologous hemopoietic cells removed prior to the injection to DMM.

With a challenge of 10^6 tumor cells, the survival rate increased from 5 (groups 12 + 13) to 44% (group 14; $P < 0.01$) after treatment with lethal DMM and autologous bone marrow. Inferior results were obtained when the DMM was applied later than one day after the tumour challenge. With a delay of nine days, a cure rate of 27% ($P < 0.1$) was secured (group 16).

No survival occurred if autologous cells were withheld after DMM. Panels of 6 mice challenged with 10^4, 10^5, 5×10^5 and 10^6 lymphoma cells, amputated on Dl (with 10^6 cells also on D3 and D9), treated with 18.5 mg/kg DMM, but not reinfused on the following day with autologous cells, died uniformly before D20 without evidence of tumors (not entered in the table).

Without DMM, tumor fatalities were not influenced by amputation and reinfusion of autologous cells (groups 2 vs 3, 5 vs 6, 12 vs 13). Accordingly, mice without and with amputation and marrow reinfusion could be combined to serve as tumor controls not treated with DMM.

Comparison of syngeneic and autologous restoration

A comparison of the results obtained with syngeneic and autologous restoration in mice carrying the YBA lymphoma and treated with DMM is presented in Table 3. It shows that both the proportion of mice surviving DMM toxicity owing to the hemopoietic inoculum (55–100%) as well as the proportion of survivors with tumor-free remissions (73–100%) are essentially similar with either syngeneic or autologous hemopoietic reconstitution.

Therapy of Meth A sarcoma with DMM and autologous marrow

If the described experimental protocol was applied to Balb/c mice carrying the Meth A sarcoma, comparable results were obtained (Table 4). Both doses of DMM (18.5 and 24.5 mg/kg) and autologous marrow approximately doubled the survival rate after a challenge with 10^6 Meth A sarcoma cells (group 1 vs group 4: $P < 0.005$; group 1 vs group 7: $P < 0.05$). Since 18.5 mg/kg DMM were fatal to only 17% of the mice (group 2) but 24.5 mg/kg to 100% (group 5), the full potential of superdosis chemotherapy with marrow rescue was tested only with the latter dose. It increased the survival against an LD_{90} tumor challenge with 10^7 cells from 10 (group 8) to 55% (group 10; $P < 0.025$). With 18.5 mg/kg DMM only 25% of the mice challenged with 10^7 Meth A cells survived (group 9). The experiment demonstrates also the effectiveness of autologous marrow rescue in Balb/c mice after a lethal dose of DMM (group 6 vs 5).

Enhanced toxicity of DMM in tumor bearing mice

In several experiments involving tumor chemotherapy, it was noted that a significantly higher proportion of mice carrying the Moloney lymphoma as compared to tumor-free

Table 3. Survival of CBA mice challenged subcutaneously with the YBA lymphoma, treated with 18.5 mg/kg *DMM and syngeneic or autologous hemopoietic restoration*

Restoration	No. of tumor cells	Proportion of mice surviving DMM and hemopoietic graft (survivors) at D20	Proportion of survivors remaining tumor-free	Proportion of survivors succumbing to tumor
Syngeneic	10^6	15/18	11/15	4/15
Autologous	10^4	14/20	14/14	0/14
Autologous	10^5	8/8	8/8	0/8
Autologous	5×10^5	10/11	8/10	2/10
Autologous	10^6	5/9	4/5	1/5

Drawing upon values from Table 1 (syngeneic restoration, combined groups 3 and 6) and Table 2 (autologous restoration) the survival rate of mice after tumor challenge, therapy with DMM (on Dl) and either syngeneic or autologous hemopoietic restoration is compared. Nominators give the No. of surviving mice, denominators the total No. of mice per group. The two columns on the right relate to the mice (survivors) which were successfully grafted on D2 and rescued from DMM toxicity.

Table 4. Survival of Balb/c mice challenged subcutaneously on D0 with the Meth A sarcoma, amputated and treated with DMM on D2 and reinfused on D3 with autologous bone marrow

Group No.	Meth A sarcoma (No. of cells)	Amputation (D2)	DMM (mg/kg i.p.)	Reinfusion of autologous marrow (D3)	Proportion of mice surviving on D			
					10	20	30	60
1	10^6	Yes	—	Yes	20/20	20/20	12/20	9/20
2	—	No	18.5	No	5/6	5/6	5/6	5/6
3	—	Yes	18.5	Yes	10/10	10/10	9/10	9/10
4	10^6	Yes	18.5	Yes	18/18	17/18	17/18	17/18
5	—	No	24.5	No	5/6	0/6	0/6	0/6
6	—	Yes	24.5	Yes	8/10	8/10	8/10	8/10
7	10^6	Yes	24.5	Yes	13/16	13/16	13/16	13/16
8	10^7	Yes	—	Yes	10/10	10/10	2/10	1/10
9	10^7	Yes	18.5	Yes	10/12	8/12	4/12	3/12
10	10^7	Yes	24.5	Yes	13/18	13/18	13/18	10/18

Autologous bone marrow cells ($\sim 15 \times 10^6$) from one hind leg of each mouse were removed, stored overnight and reinjected by the intravenous route after 15 hr. No fatalities occurred after D50 and the experiment was terminated at D60. All surviving mice showed no evidence of tumors. The statistical significance of the differences in survival rates (chi-square test) were as follows: group 4 vs group 1: $P < 0.005$; group 7 vs group 1: $P < 0.05$ and group 10 vs group 8: $P < 0.025$.

mice succumbed to DMM in spite of similar autologous bone marrow restoration (Table 2, group 1 vs groups 4, 14, 15, 16).

Contamination of bone marrow in tumor bearing mice

Figure 1 reflects the dissemination of 10^6 YBA cells according to the route of inoculation into primary recipients. Bone marrow cells removed from the primary recipients three days after the tumor inoculation and injected intravenously into syngeneic secondary recipients caused their death according to the route by which the primary tumor cell recipients were inoculated. The intravenous injection into secondary recipients of 10×10^6 bone marrow cells from primary recipients inoculated by the intravenous, intraperitoneal or subcutaneous route caused the death of seven, five and one, respectively, out of eight secondary recipients.

A comparable survival pattern was provided by bone marrow cells from primary recipients, removed one day after the YBA cell challenge and injected into syngeneic secondary recipients treated before with a lethal dose of DMM (Fig. 2).

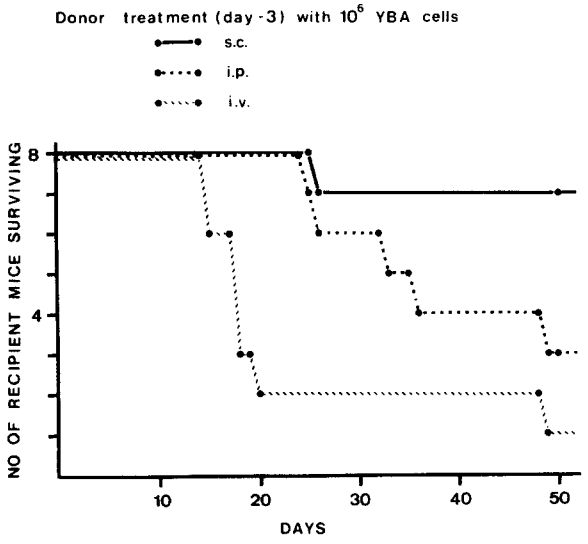

Fig. 1. Survival of CBA mice (secondary recipients) after intravenous injection of 10×10^6 bone marrow cells removed from donors (primary recipients) inoculated with 10^6 YBA cells by various routes three days earlier.

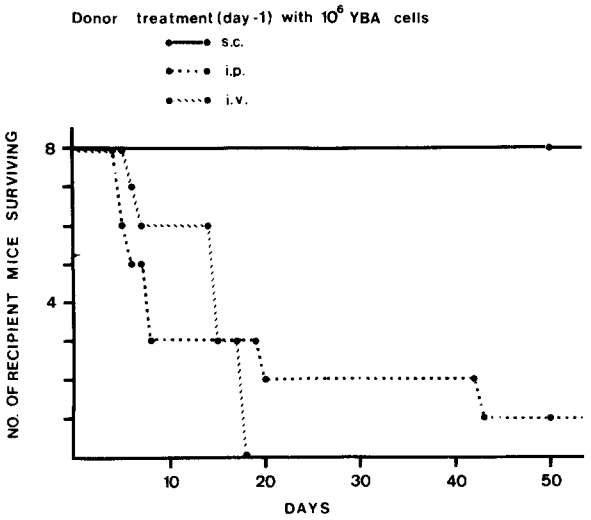

Fig. 2. Survival of CBA mice (secondary recipients) after treatment with 18.5 mg/kg DMM i.p. and intravenous injection of 10×10^6 bone marrow cells removed from donors (primary recipients) inoculated with 10^6 YBA cells by various routes one day before.

DISCUSSION

Autologous marrow grafts in conjunction with megadose cytoreductive therapy are used increasingly for clinical malignancies [3–5]. However, the experimental basis for this protocol is restricted to studies using total body irradiation to destroy canine cancers [18–20] and to one reference to superdosis chemotherapy with DMM and autologous marrow transplantation in dogs with spontaneous lymphoma [21]. Results were disappointing because of excessive DMM toxicity and limited tumor response. In contrast, DMM toxicity was more easily offset in our experiments and tumor responses were marked. It was confirmed [14] that a substantial proportion (50–80%) of mice carrying the YBA Moloney lymphoma and treated with a lethal dose of DMM combined with syngeneic bone marrow enjoy lasting tumor-free remissions (Table 1). Interestingly, a similar therapy involving syngeneic Friend- and Rauscher virus-induced leukemias in mice led to cure-rates of 50% only with spleen cells immune to the tumors [17].

We report now that both with regard to marrow rescue after lethal DMM and to the induction of tumor-free remissions, comparable results to the syngeneic protocol are obtained with lethal doses of DMM and reinfusion of autologous bone marrow removed before the injection of the drug. The autologous regimen was applied successfully to the YBA lymphoma in CBA mice and to the Meth A sarcoma in Balb/c mice. The sensitivity of the Meth A sarcoma to DMM has not been described previously and extends the therapeutic spectrum of this agent.

Therapeutic success with both tumors was related to drug dosage. With the YBA lymphoma, 18.5 mg/kg DMM secured a substantial cure rate, but 15 mg/kg ($\sim LD_{50}$) were ineffective (Table 1). Against 10^7 Meth A sarcoma cells, survival was increased five-fold with the lethal dose of 24.5 mg/kg but not significantly improved with 18.5 mg/kg, an $\sim LD_{20}$ to Balb/c mice. Our experiments thus demonstrate the rationale and viability of the concept of lethal superdosis tumor chemotherapy with marrow rescue as a potential method for eradicating tumor loads resistant to conventional chemotherapy.

A problem requiring further study is the enhanced susceptibility of tumor bearing mice to DMM, as exemplified by CBA mice challenged with 10^6 YBA lymphoma cells (Table 2, groups 4, 14, 15 and 16). Similar observations showing an reduced restorative capacity of autologous marrow in tumor bearing hosts or their increased sensitivity to cytotoxic agents have been encountered occasionally [18, 21, 22]. Although excessive DMM toxicity notwithstanding subsequent grafting of autologous marrow occurred only in tumor-bearing mice, no clear correlation with the tumor cell load was observed. This occurrence also compromised attempts to apply the described protocol to mice inoculated intraperitoneally or intravenously with tumor cells. If bone marrow fails to prevent DMM death in tumor bearing mice, likely explanations would include that a tumor factor critically impairs the restorative capacity of the marrow. We are currently investigating in a separate study this potential difficulty of superdosis tumor chemotherapy with autologous marrow rescue.

Another relevant problem in autologous marrow grafting in patients receiving superdosis chemotherapy is the contamination of the marrow with cancer and particularly leukemic cells. The question to be answered is what type and how many metastatic cancer cells carried with the autologous graft will provide further tumor spread. In preliminary experiments it was shown that three days after tumor inoculation by various routes, sufficient tumor cells reside in the marrow to be detected by secondary transplantation (Fig. 1). From experiments designed to titrate the relationship between tumor cell dose and mouse survival, it may be inferred that the marrow contained at three days after intravenous, intraperitoneal or subcutaneous tumor inoculation approximately 10^5, 10^3 and 10^1 YBA cells, respectively. This marrow contamination also compromised its restorative power in syngeneic hosts treated with a lethal dose of DMM (Fig. 2). The early death of a fraction of these mice indicates in addition some influence of the tumor on the hemopoietic potency of bone marrow. Marrow from donors carrying subcutaneous tumours retained its restorative capacity, but the demonstration of early marrow contamination is likely to compromise autologous rescue after lethal DMM in mice which have been inoculated intravenously or intraperitoneally with tumor cells.

The presented model may be useful to study autologous marrow grafting in cancer treatment and to explore problems linked to autologous restoration such as its feasibility in the presence of bone marrow metastases.

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REFERENCES

1. THOMAS ED, BUCKNER CD, FEFER A, NEIMAN PE, STORB R. Marrow transplantation in the treatment of acute leukemia. *Adv Cancer Res* 1978; **27**: 269–279.
2. STORB R, THOMAS ED. Human marrow transplantation. *Transplantn* 1979; **28**: 1.
3. GRAZE PR, GALE RP. Autotransplantation for leukemia and solid tumors. *Transplantn Proc* 1978; **10**: 177–184.
4. APPELBAUM FR, DEISSEROTH AB, GRAW RG, HERZIG GP, LEVINE AS, MAGRATH IT, PIZZO PA, POPLACK DG, ZIEGLER JL. Prolonged complete remission following high dose chemotherapy of Burkitt's lymphoma in relapse. *Cancer* 1978; **41**: 1059–1063.
5. DICKE KA, SPITZER G, VERMA D, ZANDER A, THOMSON S, MCCREDIE K, PETERS LJ, VELLEKOOP L. The use of autologous bone marrow transplantation after high-dose cytoreductive therapy in various types of malignancies in man. In BAUM SJ, LEDNEY GD, eds. *Experimental Hematology Today* New York: Springer, 1979: 221–230.
6. FLOERSHEIM GL, STORB R. Knochenmarktransplantation bei aplastischen Anämien und akuten Leukämien. *Schweiz med Wschr* 1974; **104**: 87–94
7. BUCKNER CD, DILLINGHAM LA, GIDDENS WE, THOMAS ED. Toxicologic and marrow transplantation studies in rhesus monkeys given dimethylmyleran. *Exp Hemat* 1975; **3**: 275–288.
8. STORB R, WEIDEN PL, GRAHAM TC, LERNER KG, NELSON N, THOMAS ED. Hemopoietic grafts between DLA-identical canine littermates following dimethylmyleran. *Transplantn* 1977; **24**: 349–357.
9. FEFER A, CHEEVER MA, THOMAS ED, BOYD C, RAMBERG R, GLÜCKSBERG H, BRUCKNER CD, STORB R. Dissappearance of Ph^1-positive cells in four patients with chronic granulocytic leukemia after chemotherapy, irradiation and marrow transplantation from an identical twin. *N Engl J Med* 1979; **300**: 333–337.
10. KOLB HK, STORB R, WEIDEN PL, OCHS HD, KOLB H, GRAHAM TC, FLOERSHEIM GL, THOMAS ED. Immunologic, toxicologic and marrow transplantation studies in dogs given dimethylmyleran. *Biomedicine* 1974; **20**: 341–350.
11. EINSTEIN AB, FEFER A. Effect of dimethylmyleran on cell-mediated immunity to a tumor allograft. *J Natl Cancer Inst* 1975; **55**: 1143–1146.
12. CARTER RL, CONNORS TA, WESTON BJ. Treatment of a mouse lymphoma by L-asparaginase: success depends on the host's immune response. *Int J Cancer* 1973; **11**: 345–357.
13. MOORE M, WILLIAMS DE. Contribution of host immunity to cyclophosphamide therapy of a chemically-induced murine sarcoma. *Int J Cancer* 1973; **11**: 358–368.
14. FOERSHEIM GL. Treatment of Moloney lymphoma with lethal doses of dimethylmyleran combined with injections of haemopoietic cells. Lancet 1969; **1**: 228–233.
15. TWENTYMAN PR, BLACKETT JNM. Action of cytotoxic agents on the erythroid system of the mouse. *J Natl Cancer Inst* 1970; **44**: 117–123.
16. DUNN CDR. Cell proliferation and drug resistance in the response of colony-forming units of rat bone marrow to repeated doses of cytotoxic agents. *J Natl Cancer Inst* 1972; **48**: 639–649.
17. EINSTEIN AB, CHEEVER MA, FEFER A. Use of dimethylmyleran in adoptive chemoimmunotherapy of two murine leukemias. *J. Natl Cancer Inst* 1976; **56**: 609–613.
18. WEIDEN PL, STORB R, LERNER KG, KAO GF, GRAHAM TC, THOMAS ED. Treatment of canine malignancies by 1200 R total body irradiation and autologous marrow grafts. *Exp. Hemat* 1975; **3**: 124–134.
19. WEIDEN PL, STORB R, DEEG HJ. Total body irradiation and autologous marrow transplantation as consolidation therapy for spontaneous canine lymphoma in remission. *Exp Hamat* 1979; **7**: (Suppl. 5), 160–163.
20. WEIDEN PL, STORB R, DEEG HJ, GRAHAM TC, THOMAS ED. Prolonged disease-free survival in dogs with lymphoma after total-body irradiation and autologous marrow transplantation. Consolidation of combination-chemotherapy-induced remissions. *Blood* 1979; **54**: 1039–1049.
21. WEIDEN PL, STORB R, SHULMAN H. Dimethylmyleran and autologous marrow grafting for the treatment of spontaneous canine lymphoma. *Eur J Cancer* 1977; **13**: 1411–1415.
22. KOPPER L, STEEL GG. The therapeutic response of three human tumor lines maintained in immune-suppressed mice. *Cancer Res* 1975; **35**: 2704–2713.