Eur J Cancer Clin Oncol, Vol. 18, No. 8, pp. 777–784, 1982.
Printed in Great Britain.

0277-5379/82/080777-08$03.00/0

Decreased Tolerance to Dimethyl-myleran, Cyclophosphamide and Radiation in Lymphoma-bearing Mice*

G. L. FLOERSHEIM

Laboratory for Transplantation Biology, Departments of Surgery and Research, Zentrum für Lehre und Forschung, Kantonsspital, Hebelstrasse 20, CH-4031 Basel, Switzerland

Abstract—*A lethal dose of the cytotoxic agent dimethyl-myleran (DMM) is survived by 75–100% of mice reinfused with autologous bone marrow. In mice inoculated beforehand with a syngeneic Moloney lymphoma and then treated with lethal DMM, hemopoietic restoration and survival are compromised. With subcutaneous tumour challenge, in the face of substantial early toxicity, a therapeutic effect of DMM prevails. With intravenous or intraperitoneal tumour challenge the restorative potency of the autograft and thus the therapeutic drug effect are lost since practically all animals die from DMM. The reduced tolerance of mice carrying the Moloney lymphoma applies more generally to cytotoxic measures. The toxicity from sublethal doses of DMM, cyclophosphamide (CY) and total-body irradiation (TBI) is increased in mice inoculated subcutaneously shortly before with the lymphoma cells. The LD_{50} of the three agents is reduced by up to 70% according to the number of injected tumour cells or the time allowed for their multiplication. The effect is also provided by a cell-free tumour filtrate. Since the filtrate reduces bone marrow cellularity, the decreased tolerance could be due to the impairment of hemopoiesis by a tumour factor. Two human cancers growing as xenografts in immunosuppressed mice did not increase the sensitivity to DMM.*

INTRODUCTION

IN PREVIOUS experiments, a substantial proportion of CBA mice carrying subcutaneous grafts of the syngeneic YBA Moloney lymphoma were cured by therapy with a lethal dose of dimethyl-myleran (DMM) followed by the infusion of either normal syngeneic [1] or autologous bone marrow cells [2]. However, in the latter experiments, which were designed to mimic more closely the clinical situation, the occurrence of an excessive DMM toxicity not amenable to hemopoietic compensation pointed to the possibility of a decreased tolerance of tumour-bearing animals to cytotoxic agents. The present work was devoted to a more thorough study of this potentially important phenomenon.

MATERIALS AND METHODS

Animals

Female CBA or male C3H mice (Gl. Bomholtgard, 8680 Ry, Denmark) weighing 18–24 g were used. They received Nafag pellets and sterile water *ad libitum*.

Tumours

The syngeneic YBA Moloney lymphoma was obtained from Prof. George Klein (Karolinska Institutet, Stockholm) and maintained serially by intraperitoneal injections in CBA mice. Graded tumour 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.

The cell-free tumour filtrate was prepared as follows. Suspensions of YBA cells were made up in TC 199, allowing injections of 1.5×10^6, 15×10^6 or 50×10^6 cells in 0.5 ml per mouse. The suspensions were frozen at −70°C and thawed in a water bath at 20°C three times, then passed through a 0.45-μm Millipore filter. The cell-free equivalents of the indicated numbers of YBA cells were injected in 0.5 ml intraperitoneally in the mice.

The Ewing sarcoma and the colon carcinoma

Accepted 5 March 1982.
*Supported by the Swiss National Foundation (Grants No. 3.836.76 and 3.975–0.78).

were derived from human biopsy specimens [3]. They had been passaged serially in nude mice (Iffa Credo, Lyon, France).

Drugs

Dimethyl-myleran (DMM; 1,4-dimethanesulfonoxy-1,4-dimethylbutane; dimethylbusulphan; NSC No. 23980) was kindly provided by Dr. Harry B. Wood, Division of Cancer Treatment, NCI, National Institute of Health, Bethesda, MD (U.S.A.). It was dissolved in 94% ethanol, to which warm sterile saline was added to make up for applying the desired dose in 0.2 ml per 10 g body weight. DMM was injected within five minutes after the preparation by the intraperitoneal route. The toxicity of DMM was similar in normal and anesthesized mice.

Cyclophosphamide (CY; Endoxan®; gift of Max Ritter AG, Zürich) was dissolved in distilled water and injected subcutaneously in 0.1 ml/10 g body weight.

For the human tumour xenografts, male C3H mice were prepared by the following immunosuppressive protocol. On days −3 and −1 before tumour inoculation (day 0), the mice received 135 mg/kg procarbazine hydrochloride (PCH; gift of Dr. W. Bollag, F. Hoffmann—La Roche & Co AG, Basel) and 45 mg/kg CY by two separate injections in a volume of 0.1 ml/10 g body weight. On days −2 and 0, antithymocyte serum (ATS; Microbiological Associates, U.S.A.) at the dosage of 0.15 ml/10 g body weight was injected subcutaneously [3, 4]. Tumours were grafted on day 0 about 6 hr after the last dose of ATS. No postgraft immunosuppressive treatment was used.

Irradiation

Irradiation of the mice was performed with a Linac-8 X-ray machine with dosage ranging from 100 to 1000 rads at a dose rate of 220 rad/min.

Preparation of bone marrow cells

Mice treated with lethal doses of DMM were reconstituted with autologous bone marrow. It had been obtained by amputation of one hind leg, performed approximately 2–3 hr before the administration of DMM. For the amputation, intraperitoneal nembutal anesthesia supplemented locally with 1% lidocaine was applied. The leg was amputated at the iliofemoral 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.

Bone marrow cells ($20–30 \times 10^6$) were harvested from each CBA mouse. The cells from each mouse were stored individually in 15-ml plastic tubes (Corning) in TC 199 with 2% fetal calf serum at 4°C. Viability after overnight storage was estimated with the trypan blue exclusion method and amounted to 80–90%. After about 14 hr, the autologous bone marrow cells were returned intravenously in 0.5 ml of TC 199. The controls received only TC 199.

Bone marrow cellularity and CFU

CBA mice were injected by the intraperitoneal route with 0.5 ml cell-free filtrate equivalent to 5, 15 or 50×10^6 YBA cells. The controls received only 0.5 ml TC 199. One, four, seven and twelve days later, groups of mice were sacrificed and the nucleated cells obtained from the four large bones of both hind legs were counted in a hemocytometer. The mean value of each group was used to draw Fig. 7.

Colony-forming units (CFU) were determined in four groups of CBA mice of which two had been treated similarly with the cell-free equivalent of 15×10^6 YBA cells, injected intraperitoneally at one or four days before the bone marrow cells were harvested. Aliquots of 10^4, 5×10^4, 10^5 and 5×10^5 nucleated bone marrow cells from individual mice of these two donor groups and of a TC 199-treated control group were then injected intravenously after overnight storage in TC 199 at 4°C to groups of 6 CBA recipients irradiated 2 hr before with 800 rads. Surface spleen colonies [5] were counted seven days later and the average number determined.

Statistical analysis

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

RESULTS

Compromised autologous rescue after therapy with lethal DMM in tumour-bearing mice

We have previously shown that DMM followed by autologous marrow displays a significant curative potency against two experimental mouse tumours, namely the YBA Moloney lymphoma and the Meth A sarcoma [2]. However, the therapeutic efficacy of DMM against the Moloney lymphoma was compromised by the impaired survival after lethal DMM followed by autologous bone marrow transplantation in tumour-bearing mice. While only 11% of control mice treated with 18.5 mg/kg DMM and reinjected with autologous marrow succumbed, thus demonstrating the functional capacity of reinfused autologous

marrow cells, 44% of mice subjected to this protocol but previously challenged *subcutaneously* with 10^6 Moloney lymphoma cells died within 20 days after chemotherapy and autologous transplantation. If the tumours were not treated with DMM, the mice died only between 30 and 50 days.

This observation prompted us to investigate the enhanced sensitivity of lymphoma-bearing mice to chemotherapy more closely. First, we found that the enhancement of DMM toxicity in tumour-bearing animals was more pronounced when the tumour cells were inoculated by the *intraperitoneal* or *intravenous* route. In the experiment presented in Table 1, 76% of normal CBA mice survived a lethal dose of DMM followed by autologous marrow (group 2) as compared to only 10% of mice (group 5) injected intravenously one day prior to the DMM with 10^3 Moloney lymphoma cells. The difference was statistically significant before the occurrence of tumour fatalities after day 30. Since death in mice challenged intravenously with 10^3 tumour cells but not treated with DMM occurs only after day 30 (groups 3 and 4), the early fatalities are clearly not due to tumour dissemination. The lethality of this LD_{50} tumour challenge is not influenced by the reinfusion of the autologous bone marrow (group 4). In mice challenged intravenously or intraperitoneally with 10^6 lymphoma cells, a similar acceleration of death after DMM is seen in spite of autologous reinfusion (groups 7 vs 6, 9 vs 8 and 11 vs 10).

The fate of mice challenged with 10^5 YBA cells by the intraperitoneal route is illustrated by the experiment presented in Fig. 1. The animals receiving no DMM survived on average for 30–40 days irrespective of whether the marrow from the amputated leg was reinfused or not. All mice treated with DMM and reinfused with autologous marrow died within 12 days in a similar fashion to mice treated only with DMM. This failure of marrow rescue in tumour-bearing mice after DMM was in clear contrast to the 100% survival of mice carrying no tumour but subjected to the same treatment consisting of amputation, injection of lethal DMM and reinfusion of marrow. In the mice injected with lymphoma cells by the intraperitoneal route and treated with DMM and autologous cells, no evidence of ascites was observed with the early deaths occurring before day 15. Thus, it must be concluded that mice inoculated intraperitoneally with the Moloney lymphoma are not readily amenable to marrow rescue after lethal DMM.

In contrast to experiments (1, 2) with subcutaneous tumour challenge, the observed excessive susceptibility to the cytotoxic agent prevented curative effects of lethal DMM in mice inoculated with lymphoma cells by the intravenous or intraperitoneal route.

Decreased tolerance to sublethal doses of DMM in tumour-bearing mice

Normally, CBA mice tolerate the dose of 12 mg/kg DMM. However, this dose is lethal to mice challenged one day before with 1.5×10^6 YBA cells. With subcutaneous challenge, median survival time amounted then to 17.3 ± 2.1 days (Fig. 2). This is only slightly more than the survival time of mice receiving a lethal dose of DMM (Table 1; Fig. 1). The survival time of mice which did not received DMM was 65.0 ± 22.2 days.

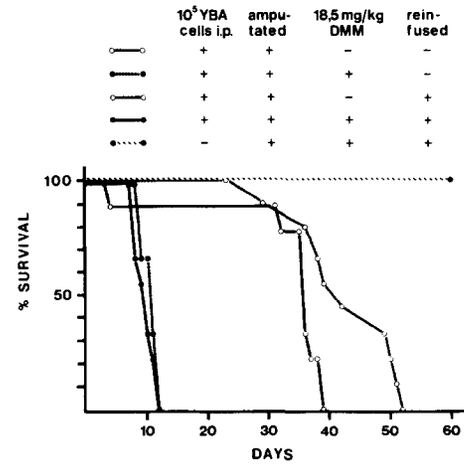

Fig. 1. Panels consisted of 10–20 female CBA mice. They were challenged intraperitoneally with 10^5 YBA cells on day 0. The amputations were carried out on day 1. DMM was administered a few hours later. Reinfusions were performed 14 hr after the amputation and survival was followed. All mice in the control group which had been amputated, treated with DMM and reinfused but not challenged with tumour cells survived.

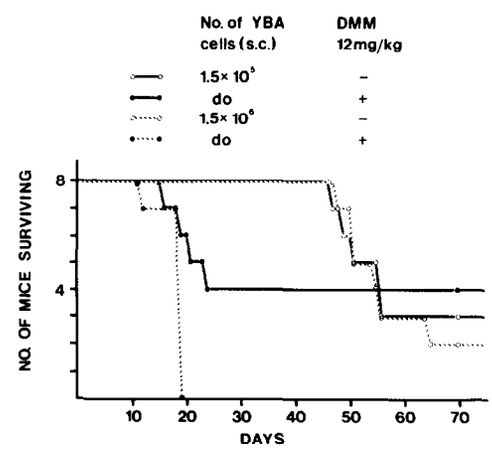

Fig. 2. Panels of 8 mice were inoculated with 1.5×10^5 or 1.5×10^6 YBA cells and treated or not treated one day later with the sublethal dose of 12 mg/kg DMM. Survival was followed.

Table 1. *Impairment of autologous marrow rescue after therapy with lethal DMM in mice challenged with the YBA lymphoma*

Group No.	Dose and route of YBA cells	Amputation on day:	DMM applied on day:	Reinfusion of marrow on day:	No. of mice	Percentage of mice surviving at day: 6	10	15	20	30	60
1	—	1	1	—	10	100	90	0			
2	—	1	1	2	67	100	94	78	76	76	76
3	10^3, i.v.	1	—	—	9	100	100	100	100	100	44
4	10^3, i.v.	1	—	2	9	100	100	100	100	100	55
5	10^3, i.v.	1	1	2	10	100	70	50	40*	10**	10
6	10^6, i.v.	1	—	2	20	100	75	45	15	5	0
7	10^6, i.v.	1	1	2	20	100	35*	5**	0		
8	10^6, i.v.	3	—	4	20	100	100	75	25	15	15
9	10^6, i.v.	3	3	4	20	85	50**	0**	0*		
10	10^6, i.p.	—	—	—	20	100	100	100	65	30	5
11	10^6, i.p.	3	3	4	30	97	40**	30**	27*	27	10

Female CBA mice were challenged on day 0 with YBA lymphoma cells and amputated on day 1 or 3. DMM (18.5 mg/kg i.p.) was injected a few hours after the amputation when the mice had recovered from the anesthesia. Autologous bone marrow cells were stored overnight and reinjected 14 hr after the amputation by the intravenous route. Mice without tumour challenge and treated with DMM but not reconstituted with autologous marrow succumbed before day 15 (group 1). Fatalities due to tumour dissemination occurred with 10^3 YBA cells after day 30 (groups 3 and 4). No fatalities were observed after day 60. The differences of the survival rates at various times were statistically significant when indicated ($^*P < 0.05$; $^{**}P < 0.01$). Survival of tumour-challenged groups receiving or not receiving DMM was compared as follows: groups 5 vs 4, 7 vs 6, 9 vs 8 and 11 vs 10.

A subcutaneous challenge with the smaller tumour dose of 1.5×10^5 YBA cells also caused early death in 50% of the mice treated with 12 mg/kg DMM (Fig. 2). In an experiment with intraperitoneal inoculation of 1.5×10^5 YBA cells (not shown), a modest therapeutic effect against this LD_{100} tumour dose prevailed since the mice escaping the initial DMM toxicity survived longer than the controls receiving only tumour cells. Also, with doses of 8.5 or 5 mg/kg of DMM in mice challenged subcutaneously with YBA cells, a moderate initial lethality occurred which was compensated by a slight therapeutic effect in the animals surviving drug toxicity.

Toxicity of DMM, CY and radiation in normal and tumour-bearing mice

As shown in Fig. 3, the LD_{50} of DMM was reduced by about 55% in mice challenged subcutaneously one day before with 10^6 YBA cells and by approximately 20% in mice challenged with 10^5 YBA cells.

Similar results were obtained with CY and total body irradiation (TBI). The LD_{50} of CY was reduced by about 30% when 1.5×10^6 YBA cells had been injected one day before and by about 70% with inoculation of this tumour dose three days earlier (Fig. 4).

With TBI, the LD_{50} was reduced from ~900 to ~550 rad after subcutaneous challenge one day before with 1.5×10^5 YBA cells and to ~350 rad with the challenge of 1.5×10^6 YBA cells (Fig. 5).

Effect of cell-free tumour filtrate on the susceptibility to DMM

As with suspensions of YBA cells, cell-free tumour filtrates increased the susceptibility to DMM. The equivalents of 1.5×10^6 or 15×10^6 YBA cells, applied by the subcutaneous route four days earlier, reduced the LD_{50} of DMM by 38% and 50% respectively (Fig. 6).

Bone marrow cellularity and stem cells after cell-free tumour filtrate

In order to identify the mechanism by which tumour-bearing mice become less tolerant to cytotoxic treatments, bone marrow cellularity was determined in normal mice after challenge with the cell-free tumour filtrate. Figure 7 shows that bone marrow cellularity was reduced by approximately 50% at four days after the injection of the filtrate. The effect was time-, but not dose- dependent within the tested range.

CFUs were determined in four groups of mice. Two were pretreated on day −1 (group 1)

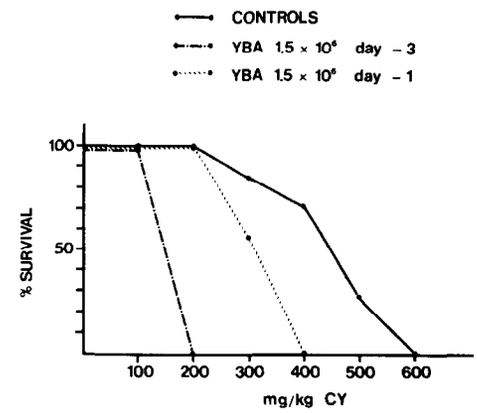

Fig. 4. Panels of 7 mice were injected intraperitoneally with cyclophosphamide at the dosages of 100, 200, 300, 400, 500 *and* 600 mg/kg. *Two panels had been challenged subcutaneously either one or three days before with* 1.5×10^6 *YBA cells. The third panel was not challenged with YBA cells.*

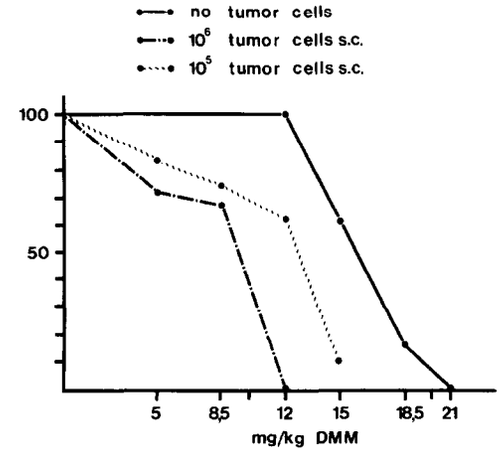

Fig. 3. Doses of 5, 8.5, 12, 15, 18.5 *and* 21 mg/kg *DMM were administered to three panels of* 8–16 *CBA mice. Two panels had been inoculated subcutaneously one day before with either* 10^5 *or* 10^6 *YBA cells, while the third panel and received no tumour cells.*

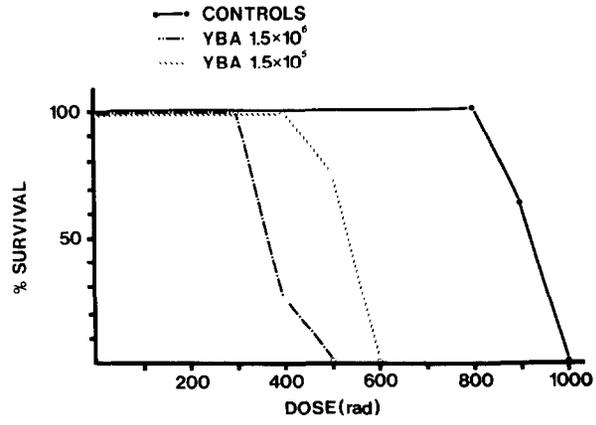

Fig. 5. Panels of 8–16 *mice received total-body irradiation with doses ranging from* 200 *to* 1000 rads. *Two panels had been injected* 24 hr *earlier by the subcutaneous route with either* 1.5×10^5 *or* 1.5×10^6 *YBA cells. The control panel did not receive YBA cells.*

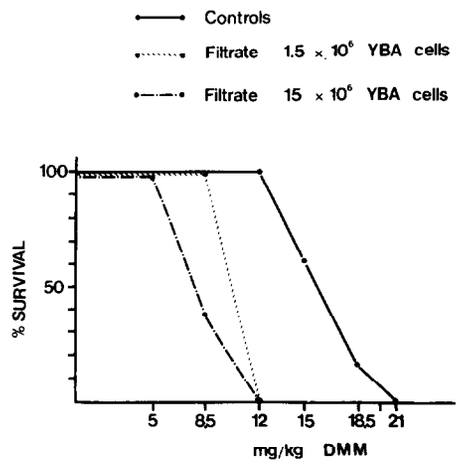

Fig. 6. Panels of 16 mice were injected with 5, 8.5, 12, 15, 18.5 and 21 mg/kg DMM. Four days earlier, two groups had been pretreated with 0.5 ml cell-free filtrate, corresponding to the equivalent of 1.5×10^6 or 15×10^6 YBA cells. The control group had not been pretreated.

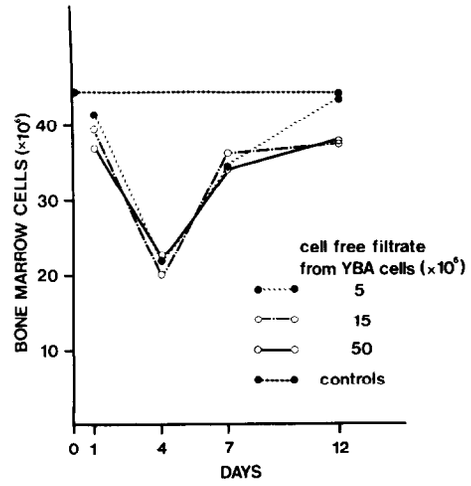

Fig. 7. Cell-free filtrates (0.5 ml) equivalent to 5, 15 or 50×10^6 YBA cells were injected intraperitoneally to groups of six female CBA mice. They were killed after one, four, seven or twelve days and the nucleated cells recovered from two tibiae and two femora were counted. Groups of six control mice received 0.5 ml TC 199 intraperitoneally. Their four bones yielded ~ 44×10^6 nucleated cells.

of −4 (group 2) before cell harvesting with the cell-free equivalent of 15×10^6 YBA cells. The other two groups were treated on day −1 (group 3) or −4 (group 4) with medium TC 199 (controls). This experiment showed that the donor mice of each group yielded similar counts of CFUs per number of injected bone marrow cells. For instance, per 10^5 bone marrow cells we found 23 (group 1), 21.7 (group 2), 22.5 (group 3) and 22.0 (group 4) CFUs. The average cellularity of two bones (femur and tibia) of one leg of these mice amounted to 26.5×10^6, 15.1×10^6, 29.8×10^6 and 30.2×10^6 cells. Accordingly, the absolute CFUs count per organ (femur and tibia) was diminished proportionally to the reduced cellularity of the organ, e.g. by approximately 50% in the mice pretreated at day −4 with the cell-free filtrate (group 2).

No decreased tolerance to DMM in mice carrying human tumour xenografts

Male C3H mice subjected to a four-day pregraft immunosuppressive protocol consisting of procarbazine, CY and ATS [3, 4], and grafted with human tumour xenografts (Ewing sarcoma and colonic carcinoma), displayed the same sensitivity to doses of DMM ranging from 5 to 21 mg/kg administered one day postgraft as controls which had been immunosuppressed but not transplanted. However, the immunosuppressive pretreatment reduced the LD_{50} of DMM in C3H mice from ~13 to ~10 mg/kg.

DISCUSSION

The reduced tolerance to DMM in mice carrying subcutaneous lymphomas leads, in spite of marrow restoration, in a substantial proportion of the animals, to an early death through hemopoietic failure which can be likened to the fatalities occurring one or two weeks after administration of lethal DMM without hemopoietic reconstitution. If the tumour challenge is applied intravenously or intraperitoneally, the survival after DMM and autologous restoration is more drastically curtailed. Fatalities occur as early as in non-restored DMM-treated mice (Fig. 1) without evidence of tumour, and clearly earlier than in mice killed by the tumour.

Since in some experiments with sublethal doses of DMM the antitumor effect of the drug was curtailed by fatalities due to hemopoietic failure, it was also investigated whether the tolerance to *sublethal* doses of DMM, CY and X-rays were decreased. This was indeed the case with all three cytotoxic measures (Figs 3–5). The increased susceptibility was not only seen in mice inoculated with intact tumour cells but also, as shown with DMM, by a cell-free tumour filtrate (Fig. 6).

We could find no report designed to study whether tumour-bearing animals display an altered tolerance to cytotoxic agents. However, incidental evidence was brought forward in experiments involving cancer-bearing animals subjected to agressive cytoreductive radiotherapy or chemotherapy. Excessive acute gastrointestinal toxicity and failure of hemopoietic recovery occurred in dogs with spontaneous malignancies treated with total body irradiation

and marrow grafts [6–8]. In dogs with spontaneous lymphoma, treated with lethal DMM and autologous marrow, severe toxicity was observed at one half of the dose of DMM required to produce comparable toxicity in normal dogs [9]. Gastrointestinal toxicity as a cause of early death can be ruled out in our experiments since mice will then die within 4–6 days and not, as we observed, within 8–15 days.

As an explanation, one might invoke impaired biotransformation of cytotoxic agents because of liver damage from cancerous metastatic infiltration. Indeed, impaired biotransformation of drugs such as zoxazolamine [10] or of CY and 6-mercaptopurine [11] has been demonstrated in carcinoma-bearing rats at 16 days after the tumour transplantation. However, liver metastasis is not likely to be contributory in our experiments at only one day after inoculation of such relatively small tumour loads as 10^3–10^6 cells. Moreover, the effect was also provided by a cell-free tumour filtrate, and the survival after X-rays was similarly reduced.

One might relate the increased toxicity to an effect of the tumour cells on the hemopoietic potential of the bone marrow. Some support for tumour-induced marrow damage is provided by the treatment of syngeneic donors with cell-free filtrate (Fig. 7). It was associated with a 50% decrease of marrow cellularity, paralleled by a proportional absolute stem cell reduction. In addition, the functional capacity of the remaining stem cells may have been impaired. In the experiments involving reinfusion of autologous marrow removed after tumour inoculation, the possible contamination of the marrow with tumour cells [2] fails to explain the early DMM-type of death occurring much before the death to full tumour challenge (Table 1; Figs 1, 2). Moreover, the objection of reinfusing tumour-contaminated marrow is excluded in the experiments involving the administration of sublethal cytotoxic treatments (Figs 3–6).

Since TBI, DMM and CY cause death by hemopoietic failure, sublethal doses of these agents must spare sufficient marrow elements to ensure survival. Our experiments suggest that the Moloney lymphoma cells release a factor which impairs the residual compensatory potential of the marrow reserve persisting after sublethal damage to an extent which is not compatible any more with life. The fact that fatalities after sublethal doses of TBI, DMM and CY in tumour-bearing mice occurred overwhelmingly between 7 and 15 days strongly indicates a marrow death.

Inhibition of normal marrow growth in agar in diffusion chambers by leukemia cells, conditioned media or extracts of these cells has been reported [12–19]. In these studies, inhibition has been shown to be directed against both committed granulopoietic and pluripotent stem cells. A recent biochemical characterization of this leukemia-associated inhibitor (LAI) from human leukemic cells is consistent with LAI being an acidic isoferritin [17]. This factor may explain the suppression of normal hemopoiesis in leukemic patients resulting in serious clinical problems from granulocytopenia, thrombocytopenia and anemia. However, our study does not permit us to exclude that the observed effect is virus-mediated rather than mediated by a soluble substance from the tumour cells.

The present work provides circumstantial evidence for this effect in the Moloney lymphoma, in addition to mice infected with the Abelson [20] and Friend virus [21]. Further, it raises the possiblility that leukemic individuals may display a decreased tolerance to X-rays and cytotoxic agents.

Further studies must elucidate whether other tumours cause this effect too. The negative results obtained with the human tumours or the Meth A sarcoma [13] are not sufficient to answer this question. They are, on the other hand and in the face of the observations of Weiden *et al.* [9], reassuring with regard to the possibility of treating human tumours with lethal doses of DMM and autologous bone marrow [4].

Acknowledgements—I gratefully acknowledge the technical assistance of Mrs. Denise Nassenstein and Mrs. Nicole Chiodetti.

REFERENCES

1. FLOERSHEIM GL. Treatment of Moloney lymphoma with lethal doses of dimethylmyleran combined with injections of haemopoietic cells. *Lancet* 1969, **i**, 228–233.
2. FLOERSHEIM GL. Treatment of murine tumours with lethal doses of dimethylmyleran and autologous bone marrow. *Eur J Cancer* 1981, **17**, 905–912.
3. FLOERSHEIM GL, NASSENSTEIN D, TORHORST J. Growth of human tumours in mice

after short-term immunosuppression with procarbazine, cyclophosphamide and antilymphocyte serum. *Transplantation* 1980, **30**, 275–280.

4. FLOERSHEIM GL, TORHORST J. Control of a human tumour (Ewing sarcoma) in mice by a single lethal dose of dimethyl-myleran and bone marrow. *Int J Cancer* 1981, **27**, 743–748.
5. TILL JE, MCCULLOUGH EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res* 1961, **14**, 213–222.
6. EPSTEIN RB, GRAHAM R, STORB R, THOMAS ED. Studies of marrow transplantation, chemotherapy and cross-circulation in canine lymphosarcoma. *Blood* 1971, **37**, 349–359.
7. 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 Hematol* 1975, **3**, 124–134.
8. WEIDEN PL, STORB R, SALE GE, GRAHAM TC, THOMAS ED. Allogeneic hematopoietic grafts after total-body irradiation in dogs with spontaneous tumours. *J Natl Cancer Inst* 1978, **61**, 353–357.
9. WEIDEN PL, STORB R, SHULMAN H, GRAHAM TC. Dimethylmyleran and autologous marrow grafting for the treatment of spontaneous canine lymphoma. *Eur J Cancer* 1977, **13**, 1411–1415.
10. GARATTINI S, DONELLI MG, SPREAFICO F. Factors affecting biotransformation and activity of antitumour drugs. *Biochem Pharmacol* 1974, **23**, Suppl. 2, 21–33.
11. BARTOSEK I, DONELLI MG, GUAITANI A, COLOMBO T, RUSSO R, GARATTINI S. Differences of cyclophosphamide and 6-mercaptopurine metabolic rates in perfused liver of normal and tumour-bearing animals. *Biochem Pharmacol* 1975, **24**, 289–290.
12. CHIYODA S, MIZOGUCHI H, KOSAKA K, TAKAKU F, MIURA Y. Influence of leukaemic cells on the colony formation of human bone marrow cells *in vitro*. *Br J Cancer* 1975, **31**, 355–358.
13. MORRIS TCM, MCNEILL TA, BRIDGES JM. Inhibition of normal human *in vitro* colony forming cells by cells from leukaemic patients. *Br J Cancer* 1975, **31**, 641–647.
14. MILLER AM, PAGE PL, HARTWELL BL, ROBINSON SH. Inhibition of growth of normal murine granulocytes by cocultured acute leukaemic cells. *Blood* 1977, **50**, 799–809.
15. QUESENBERRY PJ, RAPPEPORT JM, FOUNTEBUONI A, SULLIVAN R, ZUCKERMAN K, RYAN M. Inhibition of normal murine hematopoiesis by leukemic cells. *N Engl J Med* 1978, **299**, 71–74.
16. HANDLER ES, HANDLER EE, BACON ER. *In vitro* colony formation in bone marrow and spleen cell suspensions from rats with an acute myelogenous leukemia. *J Lab Clin Med* 1974, **840**, 249–256.
17. BROXMEYER HE, BOGNACKI J, DORNER MH, DE SOUSA M. Identification of leukemia-associated inhibitory activity as acidic isoferritins. A regulatory role for acidic isoferritins in the production of granulocytes and macrophages. *J Exp Med* 1980, **153**, 1426–1444.
18. OLOFSSON T, OLSSON J. Suppression of normal granulopoiesis *in vitro* by a leukemia-associated inhibitor (LAI) of acute and chronic leukemia. *Blood* 1980, **55**, 975–982.
19. OLOFSSON T, OLSSON J. Biochemical characterization of a leukemia-associated inhibitor (LAI) suppressing normal granulopoiesis *in vitro*. *Blood* 1980, **55**, 983–991.
20. BROXMEYER HL, RALPH P, GILBERTSON S, MARGOLIS VB. Induction of leukemia-associated inhibitory activity and bone marrow granulocyte–macrophage progenitor cell alterations during infection with Abelson virus. *Cancer Res* 1980, **40**, 3928–3933.
21. MARCELETTI J, FURMANSKI P. A murine model for the study of leukemia-associated inhibitory activity. *Blood* 1980, **56**, 134–137.