

# Tumor Proliferation and Chemotherapy in Immunosuppressed Mice<sup>1</sup>

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## ABSTRACT

The influences on host immunosuppression by treatment with cyclophosphamide (200 mg/kg), steroid (prednisolone, 12.0 mg/kg for seven doses or 235 mg/kg for one dose), and adult thymectomy on tumor growth were compared. Treatment with cyclophosphamide 24 hr prior to MOPC 104E tumor transplantation produced the greatest facilitation of tumor growth. The role of prednisolone in rendering the MOPC 104E cells more vulnerable to conventional chemotherapy was also investigated. The combination of prednisolone with melphalan added measurably to the cytotoxicity of the treatment and increased the percentage of disease-free survivors. The observed effects of prednisolone might have been due to the increase in the cycling of myeloma cells directly, or the drug may have facilitated growth of the myeloma by blocking host expansion of T-cell immunity. Alterations of the host by adult thymectomy and immunosuppression with cyclophosphamide or prednisolone led to growth facilitation of myeloma. The limited studies reported here point out the usefulness of facilitation of tumor growth to accomplish increased neoplastic cell kill and increased percentage of disease-free survivors.

## INTRODUCTION

The understanding of the action of cell cycle-specific and cell cycle-nonspecific chemotherapeutic agents and their relation to tumor cell kill is crucial to treatment of neoplasms. If a chemotherapeutic agent is cycle specific in its effects, then by properly spacing the administration of the drug, rapidly dividing neoplastic cells will be vulnerable to attack. The classical studies of Skipper *et al.* (15) emphasize the kinetics of tumor growth, including the percentage of tumor cells killed by different doses of chemotherapeutic agents. They showed a close relationship between dosage level and the percentage of a given leukemic cell population killed by an effective drug. The observation was made that the higher the growth fraction of the neoplasm, the steeper is the drug dose-response curve (15).

Based on these studies, we proposed to see if growth facilitation of MOPC 104E cells is induced *in vivo* and whether this provides a condition for greater kill of tumor cells. The approach has not been seriously considered, and there are inherent risks associated with using tumor growth facilitation as a possible mode in the treatment of cancer.

## MATERIALS AND METHODS

**Mice.** Six-week-old female BALB/c mice were purchased from Laboratory Supply Co. (Indianapolis, Ind.). The mice were maintained

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on standard laboratory chow (Wayne Feed Co., Chicago, Ill.) and water *ad libitum* until they reached a weight of 20 to 25 g.

**Tumor Line.** The MOPC 104E line is maintained as an ascites tumor by serial i.p. passage in BALB/cAnN mice. The original MOPC 104E plasmacytoma was induced by mineral oil in BALB/c mice by Dr. Michael Potter at NIH. We received our line from Dr. Potter in 1971 and have maintained the tumor in BALB/cAnN subline.

**Tumor Cell Suspension.** Ascites fluid was collected from the tumor-bearing mice and centrifuged in a clinical centrifuge, and the cell pellet was washed twice with Medium TC 199. The cells were suspended in Medium TC 199 fortified with 10% fetal calf serum for cell counts. An equal volume of 0.33% trypan blue was added to the cell suspension, and viable counts were made by the dye-exclusion method. The suspension was adjusted to contain  $1 \times 10^7$  viable tumor cells per ml. One-tenth ml ( $1 \times 10^6$  cells) of the tumor suspension was given i.v.

**Thymectomy.** Thymectomy was performed when the mice were 6 weeks old. The mice were anesthetized with pentobarbital, and the thymus was removed by vacuum extraction through a surgical incision. The incision was closed with autoclips. Thymectomized mice were given MOPC 104E 10 days postthymectomy. At the time of death, mice were autopsied for the successful removal of the thymus. Six ATx<sup>3</sup> mice and 6 control mice were used in this study.

**Murine Model.** The MOPC 104E plasmacytoma is transplantable and produces a monoclonal IgM. This monoclonal IgM has the unique characteristic of reacting with bacterial Dextran B-1355 (a generous gift from Dr. M. E. Slodki, Northern Regional Research Laboratory, United States Department of Agriculture, Peoria, Ill.). Sheep RBC conjugated with Dextran B-1355 can be used in the presence of MOPC 104E IgM and complement, thus permitting precise quantification of total-body idiotype IgM (8). Sheep RBC coated with Dextran B-1355 were used in the radial hemolysis in gel assay for the quantitation of MOPC 104E IgM. Sera from tumor-bearing mice were transferred into the wells of an agarose plate of sheep RBC coated with Dextran B-1355 and were allowed to diffuse at 5° for 22 hr, followed by lysis with complement at 37°. The area of lysis of unknown samples was compared with that of standard samples to obtain the amount of MOPC 104E IgM. MOPC 104E tumor cells per mouse were calculated from the total circulating MOPC 104E IgM measured for individual animals (7).

**Treatment with Cyclophosphamide, Prednisolone, and Melphalan.** BALB/c mice were treated with 200 mg cyclophosphamide per kg i.p. 24 hr prior to tumor implantation to immunosuppress the host. Prednisolone was given at 12 mg/kg/day for 7 days from Day 19 post-tumor transplantation. Tumor-bearing mice were treated with melphalan at 0.50, 0.25, and 0.125 mg/kg for 3 days singly or in combination with prednisolone from Day 19 post-tumor transplantation. Each treatment group consisted of 5 to 8 mice. Animals were monitored individually at 5-day intervals for circulating IgM produced by the MOPC 104E plasmacytoma.

## RESULTS

In the first study, mice were given injections of 200 mg cyclophosphamide per kg 24 hr prior to i.v. injection of MOPC 104E cells. Control mice were given tumor cells only. On Day

<sup>3</sup> The abbreviations used are: ATx, adult thymectomy; TCGF, T-cell growth factor.

19, there were 11 times as many tumor cells in the animals treated with cyclophosphamide as in the control animals. In this study, the doubling time of the MOPC 104E was 1.47 days in the immunosuppressed mice and 2.59 days in the control mice. The median survival times were 22 and 30 days, respectively. The growth of the tumor in cyclophosphamide-treated and control mice is given (Chart 1; Table 1).

In a second study, ATx was used as a means of immunosuppression. There were 3.4 times more tumor cells in the ATx group than in the control mice at 16 days after implantation. In both of these studies, immunosuppression of the host prior to tumor implantation allowed the MOPC 104E cells to grow faster. The growth curve was similar to that of cyclophosphamide-treated mice (data not shown).

In the third study, prednisolone was given to mice with established neoplasms. Treatment of MOPC 104E tumor-bearing

mice with prednisolone (12 mg/kg/day for 7 days) from Day 19 post-tumor transplantation produced a slight facilitation. The fact that less difference was seen in this experiment may be due to the late start in treatment. There were 1.6 times more tumor cells in the prednisolone-treated mice by Day 25. This study showed that when treatment was started in animals with relatively large tumors, growth facilitation occurred but was of much lower magnitude (Table 1).

A study was designed to determine the effects of melphalan on more rapidly growing as opposed to less rapidly growing tumors. A limiting dose of melphalan which produced minimal effects on the neoplasm was determined. Table 2 summarizes the data with melphalan at doses ranging from 0.5 to 0.125 mg/kg for 3 days. A progressive loss of effectiveness at the lower dosage occurred. At 0.125 mg/kg/day for 3 days, 75% of the animals had a partial response, and all animals died of disease progression within 100 days. The addition of prednisolone (12 mg/kg/day for 7 days) to the melphalan regimen improved the rate of response. In combination with the lowest dose of melphalan, a 100% response rate with 50% survival at 100 days was achieved.

Table 3 summarizes the effect of the drug treatment on the percentage of tumor cells killed. Melphalan used alone killed 73.6%, and in combination with prednisolone, tumor cell kill was increased to 96.6%. Prednisolone used alone produced no cell kill.

The results indicate that the melphalan and prednisolone combination was superior to melphalan alone by the following criteria: rate of decline of the IgM produced by the tumor; percentage of tumor cells killed *in vivo*; percentage of animals

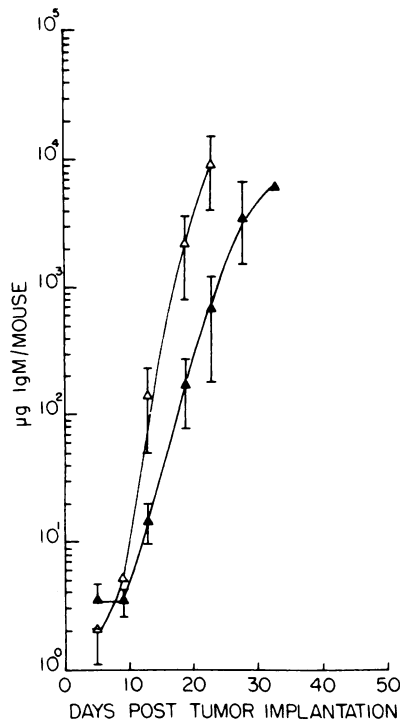


Chart 1. Growth of MOPC 104E in cyclophosphamide-treated ( $\Delta$ ) and normal BALB/c mice ( $\blacktriangle$ ). Cyclophosphamide was given at a dose of 200 mg/kg 24 hr before tumor implantation. Bars, S.E.

Table 1  
Growth of MOPC 104E in immunosuppressed mice

Treatment groups	No. of tumor cells $\times 10^6$ /mouse <sup>a</sup>	Increase over control
Cyclophosphamide	730 $\pm$ 176 <sup>b</sup>	11.1
Untreated control	65.5 $\pm$ 16	
ATx	1800 $\pm$ 530 <sup>c</sup>	3.4
Nonthymectomized control	530 $\pm$ 90	
Prednisolone	4100 $\pm$ 160 <sup>d, e</sup>	1.6
Untreated control	2540 $\pm$ 930	

<sup>a</sup> Calculated from the IgM measurement taken 19 days after tumor implantation.  
<sup>b</sup> Average/group  $\pm$  S.E.  
<sup>c</sup> Number of tumor cells/mouse at 16 days after tumor implantation.  
<sup>d</sup> Number of tumor cells/mouse at 25 days after tumor implantation.  
<sup>e</sup> Prednisolone, 12 mg/kg/day for 7 days was administered 19 days after tumor implantation.

Table 2  
Treatment of MOPC 104E myeloma with melphalan singly and in combination with prednisolone

Drugs	Dose (mg/kg)	% of mice with 50% reduction of IgM	% of survivors <sup>a</sup>
Melphalan	0.500 $\times$ 3	100	100
	0.250 $\times$ 3	100	75
	0.125 $\times$ 3	75	0
Prednisolone	12.0 $\times$ 7	0	0
Melphalan + prednisolone	0.125 $\times$ 3	100	50
	12.0 $\times$ 7		
Untreated		0	0

<sup>a</sup> No clinical evidence of disease at  $\sim$ 100 days post-tumor transplantation.

Table 3

Summary of the effects of melphalan and prednisolone on MOPC 104E myeloma tumor based on a maximum number of  $2 \times 10^9$  cells/mouse  
 For comparative purposes, the calculations were based on a maximum number of  $2 \times 10^9$  tumor cells/mouse at the time tumor cell kill started.

Drugs (dosage and schedule)	% of tumor cells killed	No. of cells killed $\times 10^9$	No. of cells remaining $\times 10^6$
Melphalan, 0.125 mg/kg for 3 doses	73.60 $\pm$ 10.72 <sup>a</sup>	1.472	528.0
Melphalan + prednisolone	96.62 $\pm$ 1.77	1.932	67.6
Prednisolone, 12 mg/kg every day for 7 days	0	0	2000.0

<sup>a</sup> Mean  $\pm$  S.E.

showing >50% reduction in tumor size; and the number of animals surviving at 100 days or more.

## DISCUSSION

Previous work from this laboratory demonstrated that the MOPC 104E model can be used to test the effects of drugs used against human myeloma (10) and is a useful model in delineating possible effects seen in humans (4, 9). We have also shown that the MOPC 104E clone is maintained in remission for longer than 200 days with effective chemotherapy (8). These results imply that the plateau phase brought about by chemotherapy indicates regulation of myeloma clones by the host. Studies by Lubet and Carlson (13), Abbas (1), and Wang *et al.* (18) have shown that host resistance can be induced against the MOPC 104E clone.

The MOPC 104E plasmacytoma model was used to determine if prednisolone adds measurably to the cytotoxic action of melphalan. The rate of decline of tumor IgM, the percentage of cells killed, the response rate, and the percentage of survival were better with the addition of prednisolone than with melphalan alone. Prednisolone by itself showed little effect on the tumor and in fact may have suppressed host effector function and indirectly enhanced growth of the tumor. Melphalan combined with prednisolone produced greater tumor cell kill, resulting in more survivors that lived beyond 100 days.

One reason for the synergistic effect with prednisolone could be explained by the fact that prednisolone acts directly on myeloma cells and increases the growth fraction of the MOPC 104E population. Or it may be that prednisolone, in causing immunosuppression of the host, is indirectly responsible for more cells dividing and inducing a higher growth fraction.

Early studies by Lubet and Carlson (11, 12) showed that in immunosuppressed animals, chemotherapy was unable to lead to eradication of MOPC 104E. These studies demonstrated that host mechanisms play a role in the destruction of the plasmacytoma. If the host has an inherent capacity to resist the neoplasm, then immunosuppression of the host prior to implantation of the MOPC 104E should facilitate growth.

In our own studies, mice given injections of 200 mg cyclophosphamide per kg 1 day prior to tumor transplantation facilitated growth of tumor. In the cyclophosphamide-pretreated animals, there were 11 times more tumor cells than in control mice, and the tumor cells were doubling at a faster rate than in controls. Animals in which tumor growth was more rapid died earlier.

To establish that immunosuppression of the host may be important to the increased rate of MOPC 104E growth, mice received ATx and were given myeloma. ATx resulted in partial suppression of the effector function. Tumor cells grew 3.4 times faster in these mice. These results are consistent with previous reports (11-13) that suppression of the host effector function could negate the effects of chemotherapy and cause rapid growth of MOPC 104E in the host.

The mechanism of immunosuppression by glucosteroids operates through T-cell proliferation. Glucosteroid suppresses leukocyte-activating factor production and exerts its effect on the macrophage and the T-cell subsets that are involved in the production of TCGF. The inhibition of TCGF production leads to prevention of TCGF-mediated T-cell proliferation and lympholysis (16).

The use of a cell cycle-specific agent during the phase of growth facilitation may augment cell kill. Melphalan inhibits cell multiplication by blocking the cell cycle in prophase (3). Cooper and Topping (5), cited by Furner and Brown (6), found that the melphalan-sensitive Yoshida ascites tumor showed a marked fall in mitotic index with accumulation of cells in G<sub>2</sub> at cell death 24 to 72 hr after treatment. A resistant Yoshida line showed only slight mitotic inhibition and a possible recovery phase 24 to 30 hr after treatment with no evidence of cellular damage.

Flow microfluorometry studies by Tobey and Crissman (17) indicate that treatment of cells with alkylating agents such as 1,3-bis(2-chloroethyl)-1-nitrosourea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, or *trans*-1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea causes preferential accumulation in the G<sub>2</sub> period. Barlogie *et al.* (2) showed that melphalan arrested human lymphoma cells in G<sub>2</sub> if the cells were exposed to the drug when they were in G<sub>1</sub> or early S phase. Although the modes of action of the alkylating agents may be different, the arrest of treated cells in the G<sub>2</sub> phase appears to be a common feature. The greater the potential of a drug to induce chromosome aberrations and damage to DNA, the higher is the frequency of G<sub>2</sub> arrest (14).

It is apparent that with the low dose of melphalan used in combination with prednisolone, greater cell kill (96.6%) is achieved than with melphalan alone (73.6%). These results are consistent with the view that in properly staged tumors, facilitation of tumor growth will cause temporarily resistant cells (G<sub>0</sub>) and, possibly, clones of drug-resistant cells to replicate during exposure to drugs. The increased susceptibility of this cycling population to phase-specific agent will permit a greater total reduction in tumor burden in animals carrying large and/or small metastatic foci of noncycling cells.

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