ANTITUMOR EFFECT OF BACTOBOLIN AND ITS INFLUENCE ON MOUSE IMMUNE SYSTEM AND HEMATOPOIETIC CELLS

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Bactobolin prolonged survival period of mice bearing leukemia L-1210 in various dose schedules. The administration of bactobolin before or at time of immunization with sheep red blood cells (SRBC) did not affect antibody formation and delayed-type hypersensitivity (DTH) to SRBC. The administration after immunization suppressed antibody formation markedly but not DTH response. Bactobolin showed stronger suppressive action on antibody formation *in vitro* than mitomycin C. Bactobolin did not reduce establishment of tumor immunity which was mediated by T cells and macrophages. Comparing to other antitumor antibiotics which were effective against L-1210, bactobolin did not affect phagocytosis of mouse peritoneal macrophages. It has an extremely low toxicity to mouse spleen cells treated by concanavalin A (Con A) and lipopolysaccharide (LPS). It did not affect colony formation of mouse bone marrow cells in the presence of LPS-induced colony stimulating factor. The administration of bactobolin did not reduce the number of leucocytes in peripheral blood. From these results, the usefulness of bactobolin in the treatment of cancer was discussed.

Bactobolin was isolated from culture filtrates of *Pseudomonas* sp. and its structure was determined[1]. It is an antitumor antibiotic which prolongs the survival period of mice bearing leukemia[1,2]. In this paper, we report the antitumor effect of bactobolin on murine transplantable tumors, and its influence on immune responses and defense mechanisms.

Materials and Methods

Mice and Tumors

CDF_1 mice (Balb/c × DBA/2, female 8 ~ 10 weeks old) were obtained from the Institute of Medical Science, University of Tokyo.

C57BL/6 mice (8 weeks old, female) and ICR mice (female, 6 weeks old) were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka, Japan. These mice were housed in plastic filter top cages and fed sterilized mouse pellet (FR-1, Funabashi Farm Co. Ltd., Chiba, Japan) and water *ad libitum*.

Mouse leukemia L-1210 was transplanted every 7 days to CDF_1 mice intraperitoneally and 10^5 L-1210 cells were inoculated intraperitoneally for the test of antitumor effect. IMC carcinoma[3] was maintained in ascitic tumor in CDF_1 mice by intraperitoneal transplantation every 7 days.

Mouse T cell-leukemia EL-4 was kindly supplied by Dr. T. TOKUNAGA, NIH, Japan and maintained in C57BL/6 mice by intraperitoneal transplantation every 7 days. EHRLICH carcinoma was maintained in ICR mice by intraperitoneal transplantation every 7 days.

Bactobolin and Other Antitumor Antibiotics

Bactobolin was prepared as reported previously[1] and dissolved in saline for animal experiments or in cell culture medium. Mitomycin C (Kyowa Hakko Co. Ltd., Tokyo, Japan), adriamycin, cyclophosphamide (Endoxan, Shionogi and Co. Ltd., Osaka, Japan) and bleomycin (Nippon Kayaku Co.

Ltd., Tokyo, Japan) were dissolved in saline or cell culture medium. A desired dose of these drugs dissolved in 0.25 ml was administered to mouse intraperitoneally and a desired concentration dissolved in 0.05 ml was added to 1 ml of culture.

Antibody-formation and Delayed-type Hypersensitivity to SRBC

CDF_1 mice were immunized with 10^8 sheep red blood cells (SRBC) (Funabashi Farm Co. Ltd., Chiba, Japan), 4 days thereafter, antibody-forming cells in mouse spleen were enumerated in terms of plaque-forming cells (PFC) by hemolytic plaque technique[4]. Antibody-formation to SRBC in spleen cell cultures was tested by the methods described by MISHELL and DUTTON[5] and by CLICK *et al.*[6] A test substance was added at start of the culture and PFC was enumerated 4 days thereafter. DTH to SRBC was tested to see the influence of bactobolin on cell-mediated immune response. As described previously[3,7], CDF_1 mice were immunized by subcutaneous injection of 10^8 SRBC to footpad of hind paw, 4 days thereafter, the same number of SRBC was injected to the other footpad, 24 hours thereafter, the resulting edema was measured by a caliper. Bactobolin was injected at various timings intraperitoneally.

Establishment of Specific Tumor Immunity to IMC Carcinoma by Immunization with Auromomycin-treated IMC Carcinoma Cells

CDF_1 mice were immunized by subcutaneous injection of mixture of 0.02 μg of auromomycin[8] and 10^6 IMC carcinoma cells to footpad and bactobolin or other antitumor antibiotics were given intraperitoneally daily for 5 days from 1 day after the immunization. Fourteen days thereafter, 10^6 IMC carcinoma cells were inoculated to the other footpad subcutaneously and the size of the tumor was recorded in 0.1 mm by a caliper.

Blastogenesis of Mouse Spleen Cells Stimulated by Concanavalin A (Con A) and Lipopolysaccharide (LPS)

According to the method reported previously[9], spleen cells were collected from CDF_1 mice and suspended in RPMI 1640 containing 10% fetal calf serum (Lot 90380, Microbiological Associate, Bethesda, Md., U.S.A.) at 1×10^6 cells/ml. The cell suspension (0.2 ml) was placed in a well of Microtest plate (Falcon 3042, Div. Becton, Dickinson and Co., Oxnard, Calif., U.S.A.). Con A, 0.1 μg (Concanavalin A, Pharmacia Fine Chemicals AB, Uppsala, Sweden) or 0.5 μg of LPS (Lipopolysaccharide, *Escherichia coli* 0111, Difco Laboratories, Detroit, Mich., U.S.A.) in 10 μl and each dose of bactobolin or other antitumor substances in 10 μl was added at the start of the culture. Each group was consisted of triplicate cultures. The plate was incubated at 37°C for 3 days in a fully humidified atmosphere of 5% CO_2 and air. Eighteen hours before assay, 0.1 μCi/well of ^{3}H-thymidine (^{3}H-TdR, 6 ^{3}H-thymidine NET-355, New England Nuclear, Boston, Mass., U.S.A.) in 10 μl was added to each culture and the incorporation of ^{3}H-TdR into cultured cells was determined by the procedure described previously[9].

Production of Haematopoietic Colony-forming Cells *in vitro* (CFU-C)

Production of CFU-C was tested according to the methods described by METCALF[10] and STANLEY *et al.*[11] Bone marrow stem cells were collected from femora of CDF_1 mice and suspended in α-minimum essential medium (α-MEM, Flow Laboratories, McLean, Va., U.S.A.) including 10% fetal calf serum (Microbiological Associates), 5% horse serum (GIBCO Laboratories, Grand Island, N.Y., U.S.A.), 10% tripticase soy broth (Difco Laboratories), 75 μg/ml of DEAE dextran (Pharmacia), 5×10^{-5} M of 2-mercaptoethanol, 20 μg/ml of asparagine, 290 μg/ml of glutamine and 0.3% Bacto-Agar (Difco Laboratories) at 7.5×10^4 cells/ml. One ml of cell suspension was placed in a plastic dish (Falcon 3001) and 0.1 ml of a diluted LPS-injected mouse serum as colony-stimulating factor[10] and 0.05 ml of bactobolin or other antitumor substances were added.

The dishes were incubated at 37°C in a fully humidified atmosphere of 10% CO_2 in air. Each group was consisted of triplicate cultures. Eight days thereafter, colonies were counted at 40 magnification using a microscope.

Phagocytosis of Yeasts by Mouse Peritoneal Macrophages *in vitro*

CDF_1 mice were injected intraperitoneally with 1 ml of thioglycollate broth (Eiken Chemical Co.

Ltd., Tokyo, Japan), 4 days thereafter, peritoneal macrophages were collected by washing peritoneal cavity with 5 ml of Dulbecco phosphate buffered saline (DPBS, GIBCO). The cells were washed and suspended in DPBS at 5×10^5 cells. One ml of the cell suspension was placed in 35 mm plastic dish (Falcon 3001) and incubated at 37°C in 5% CO_2 for 1.5 hours. After the incubation, dishes were washed with DPBS and non-adherent cells were removed. Bactobolin or other antitumor substances at various concentrations in 1 ml of DPBS was added to the macrophage-monolayer on dish and incubated for 30 minutes. After the incubation, the dishes were washed with DPBS thoroughly and 1 ml of DPBS containing 0.2 ml of heat-inactivated *Saccharomyces cerevisiae* at 3.75×10^7 cells/ml was added and incubated at 37°C in 5% CO_2. After 45 minutes, the dishes were washed with DPBS thoroughly and stained with MAY-GRÜNWALD and GIEMSA solution. Quantitative evaluation of phagocytic cells which ingested yeasts was performed by counting 200 macrophages using a microscope at 400 magnification.

Determination of the Number of Leucocytes in Peripheral Venous Blood of Mice after the Administration of Antitumor Substances

Bactobolin and other antitumor substances were injected daily for 5 days intraperitoneally and after the injection, 20 μl of venous blood was collected from orbital venous plexus by puncture with sterile glass capillary every 2 or 3 days in the first week and every 4 or 5 days in the second week after the last injection. The blood was diluted with ISOTON (Coulter Diagnostics, Hialeah, Fla., U.S.A.) containing ZAP-OGLOBIN (Coulter Diagnostics) and leucocytes were counted by a Coulter counter (Coulter Diagnostics).

Results

Antitumor Effect of Bactobolin on Murine Transplantable Tumors

Mice that were implanted 10^5 L-1210 cells intraperitoneally, 24 hours later, received various doses of bactobolin daily for 10 days, or on days, 1, 3 and 5 or on day 1 after the inoculation. Results are shown in Table 1. The administration of bactobolin 2.5~0.15 mg/kg/day daily for 10 days prolonged the survival period. The administration of 5 mg/kg/day daily for 3 or 4 days caused reduction in body weight and death of mice. The LD_{50} of bactobolin was estimated to be

Table 1. Antitumor effect of bactobolin on L-1210.

	mg/kg/day	Schedule[1]	M.S.D.[2] (T/C)	T/C %
A	2.5	1~10	18.4	236
	1.2	〃	17.0	218
	0.6	〃	28.3	363
	0.3	〃	15.2	195
	0.15	〃	10.4	133
	0.08	〃	8.4	108
B	5.0	1, 3, 5	16.3	209
	2.5	〃	14.2	182
	1.2	〃	12.8	164
	0.6	〃	10.5	135
C	5.0	1	11.7	150
	2.5	〃	11.3	145
	1.2	〃	10.0	128
	0.6	〃	10.2	131
	0	—	7.8	100

1) Bactobolin was administered to CDF_1 mice (female, 10 weeks old) from 1 to 10 days (A), 1, 3, 5 days (B) and 1 day (C) after inoculation of 10^5 L-1210 cells intraperitoneally.
2) Mean survival days of 5 mice.

Table 2. Antitumor effect of bactobolin against murine transplantable ascitic tumors.

mg/kg/day[1]	T/C %			
	L-1210	EL-4	IMC Ca.	EHRLICH
2.5	236	152	206	215
0.6	363	112	158	219
0.15	133	101	127	103
Implantation[2] of No. of cells	1×10^5	1×10^5	1×10^6	2×10^6
Mice strain	CDF_1	C57BL/6	CDF_1	ICR

1) Each dose of bactobolin was administered intraperitoneally daily for 10 days from 1 day after the inoculation of tumor cells.
2) The strain of mice used for the test.

6.25~12.5 mg/kg in CDF_1 mice by intraperitoneal route. The maximum tolerated dose, the most effective dose and the minimum effective dose were 2.5 mg, 0.6 mg and 0.15 mg/kg/day respectively. On a dosage schedule of three injections of every 2 days, mice tolerated 5 mg/kg/day and this schedule was effective. The minimum effective dose was 0.6 mg/kg/day. The effect of a single injection of bactobolin on L-1210 mouse leukemia was also examined. As shown in Table 1-C, the maximum and minimum effective doses were the same as those on the schedule of three injections every 2 days, although the latter schedule was significantly more effective.

These results indicate that bactobolin can prolong the survival period of L-1210 bearing mice with a high therapeutic index and that the frequent administration of a low dose was appeared to be more effective. However, a dose approaching the lethal dose could be administered, if this was given at specific intervals.

Effects of bactobolin on a variety of murine tumors were examined. As shown in Table 2, in tests against L-1210, EL-4, IMC carcinoma and EHRLICH carcinoma, L-1210 was most susceptible to bactobolin. On the contrary, mouse leukemia EL-4 was most resistant. Since EL-4 is known to be a T cell-leukemia, our data suggests that T cells might be more resistant to bactobolin than B cells and other lymphoid cells.

Influence of Bactobolin on Immune Responses to SRBC in Mice

In order to determine the effect of bactobolin on immune responses, antibody formation and delayed-type hypersensitivity (DTH) to sheep red blood cells (SRBC) was tested. Bactobolin at various doses was injected to mice daily for 4 days and 24 hours thereafter, mice were immunized with 10^8 SRBC intravenously for antibody formation or subcutaneously to the hind footpad for DTH test. Four days later, antibody formation was measured by hemolytic plaque assay and DTH was elicited by the injection of 10^8 SRBC to the other hind footpad. The resulting edema was measured 24 hours later. As shown in Table 3-A, the administration of 2.5 mg/kg/day, which was the dose exhibiting a strong antitumor activity, showed no significant depression on both immune-responses in treated mice.

Table 3. Effect of bactobolin on immune responses to SRBC in mice.

	Bactobolin administered	D.T.H.[1] increase of footpad thickness (×0.1 mm)	Ab-formation[2] PFC $\times 10^3$/spleen
A[3]	2.5 mg/kg, for 4 days, i.p.	9.1±0.7	337±28
	0.25 〃 〃	12.0±1.2	418±39
	0.025 〃 〃	12.5±1.7	617±60
B[4]	5.0 mg/kg, i.p.	10.2±0.8	756±14
	0.5 〃	14.9±0.4	585±19
	0.05 〃	12.0±0.9	570±31
	0.005 〃	11.2±1.0	304±53
	0	10.3±1.5	339±26

1) CDF_1 mice (female, 10 weeks old) were immunized with 10^8 SRBC to hind footpad s.c., 4 days thereafter, the same number of SRBC was given for elicitation to the other footpad and 24 hours thereafter, the result was recorded.
2) CDF_1 mice were immunized with 10^8 SRBC i.v. and 4 days thereafter, PFC was counted.
3) Bactobolin was administered once a day daily for 4 days before immunization.
4) Bactobolin was administered once at the time of immunization.

The administration of a single dose of bactobolin at the time of immunization was tested. Results are shown in Table 3-B. Although 5 mg/kg, the dose close to the lethal dose were given to mice intraperitoneally, both types of immune responses were not affected. The administration of 5 mg/kg or 0.5 mg/kg showed a stimulatory effect on antibody formation and DTH respectively. The stimulatory effect was observed only in a narrow dose range of bactobolin.

Results indicate that the single or multiple doses of bactobolin to mice prior to or at the time of immunization dose not affect immune responses even in doses close to lethal dose.

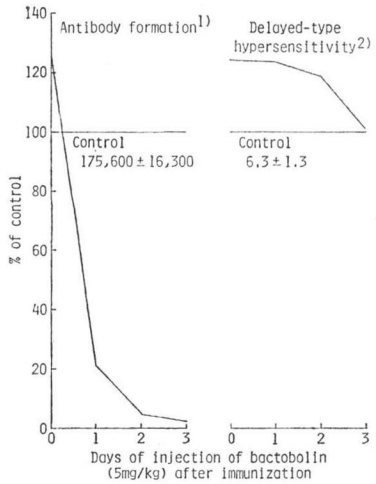
To determine whether the adminstration of bactobolin after immunization affects immune responses, mice were immunized with SRBC and 5 mg/kg of bactobolin was administered intraperitoneally on each day after immunization. Antibody formation and DTH response were examined.

Results are shown in Fig. 1. DTH response was not affected by the administration of a high dose of bactobolin given after immunization. On the other hand, although the number of antibody-forming cells was not reduced by the administration of bactobolin at the time of immunization, bactobolin administered on 1, 2 or 3 days after immunization strongly suppressed the production of antibody-forming cells in mouse spleens. These results indicate that bactobolin does not affect T cell-mediated immune responses, but inhibits the differentiation of B cells to plasma cells and/or plasma cells.

The influence of bactobolin on antibody formation in spleen cell culture was compared to other antitumor antibiotics added at the start of culture. As shown in Fig. 2, bactobolin showed a stronger suppressive effect than mitomycin C. The ID_{50} of each antitumor antibiotic was as follows: bactobolin, 0.02 μg/ml; mitomycin C, 0.11 μg/ml; adriamycin, 0.016 μg/ml; bleomycin, >1 μg/ml. In this experiment, viable cell counts in these cultures on day 4 were determined by trypan blue dye exclusion. The addition of 1 μg/ml of bactobolin, 0.01 μg/ml of mitomycin C, 0.1 μg/ml of adriamycin, or 1 μg/ml of bleomycin reduced the number of viable cells by more than 50% of control. However, the addition of lower concentrations of these antibiotics did not reduce the number of viable cells. It would appear that bactobolin has the strong-

Fig. 1. Effect of bactobolin on immune responses to SRBC.

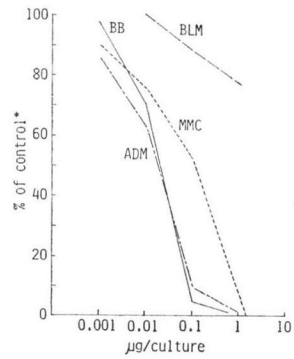
Injection of bactobolin at a different time after immunization.



1) 4 days result after immunization.
2) 24 hours results after eliciting injection on 4 days after immunization.

Fig. 2. Inhibitory effects of antitumor substances on antibody formation to SRBC *in vitro*.

BB, bactobolin; BLM, bleomycin; ADM, adriamycin; MMC, mitomycin C.



* The number of PFC of control was 2,010/culture.

est activity to suppress antibody formation *in vitro* when compared to the other tested antitumor antibiotics.

Influence of Bactobolin on the Establishment of Tumor Immunity

The above results suggested that bactobolin did not affect T cell-mediated immune responses. Studies were performed to determine whether the antitumor substances which are effective on leukemia, suppress the establishment of tumor immunity mediated by T cells and macrophages. The effect of bactobolin was tested on tumor immunity induced by immunization with a syngeneic tumor, IMC carcinoma cells treated with auromomycin in mice. The establishment of tumor immunity with auromomycin in this system has been determined to be due to T cell- and macrophage-mediated immune response[12].

CDF_1 mice were immunized by subcutaneous injection of 10^6 IMC carcinoma cells mixed with auromomycin to right hind footpad and 25 mg/kg/day of bactobolin, 50 mg/kg/day of cyclophosphamide, 1.2 mg/kg/day of mitomycin C or 5 mg/kg/day of bleomycin was given daily for 5 days after the immunization. The doses of these test substances employed in this experiment was approximately 1/4 or 1/8 LD_{50} by the intraperitoneal route, except for bleomycin. Seven days later, mice were challenged by injection with 10^6 IMC carcinoma cells to the other hind footpad subcutaneously. Thereafter, the tumor size was measured by a caliper and recorded in 0.1 mm. The results are shown in Fig. 3. Mice that were immunized and treated with saline were markedly resistant to the transplantation of IMC carcinoma cells. However, treatment with cyclophosphamide or mitomycin C suppressed this immunity. In the case of bactobolin and a low dose of bleomycin, the establishment of the immunity was not suppressed. The results

Fig. 3. Effects of antitumor substances on the establishment of tumor immunity.

CY, cyclophosphamide; MMC, mitomycin C; BLM, bleomycin; BB, bactobolin.

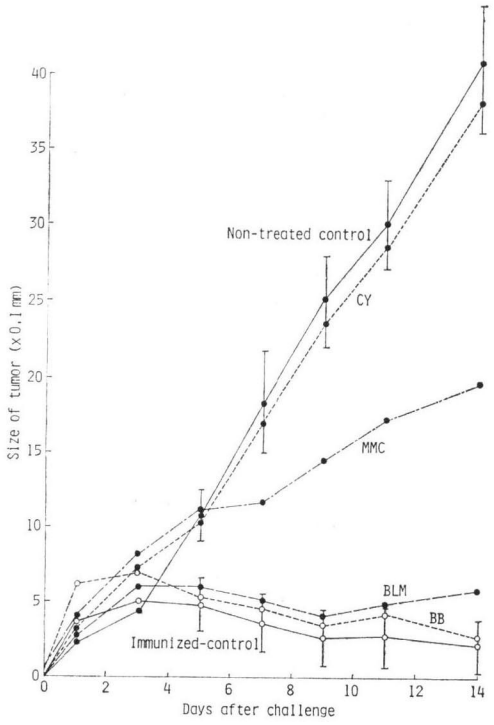
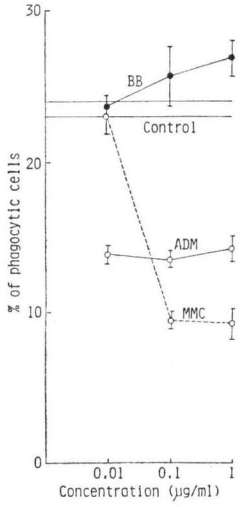


Fig. 4. Influence of antitumor substances on phagocytosis activity of mouse peritoneal macrophages *in vitro*.

BB, bactobolin; ADM, adriamycin; MMC, mitomycin C.



of these tests indicate that among the substances showing anti L-1210 activity, bactobolin, even at high dose, does not suppress the establishment of tumor immunity which is mediated by T cells and macrophages.

Influence of Bactobolin on Phagocytosis of Yeasts by Mouse Peritoneal Macrophages *in vitro*

The effect of bactobolin on phagocytosis of mouse peritoneal macrophages was studied. Monolayer cultures of peritoneal macrophages were incubated with bactobolin, adriamycin or mitomycin C for 30 minutes and washed, and phagocytosis of yeasts was examined in each culture. As shown in Fig. 4, a short time treatment of cultures with 0.1 μg/ml of adriamycin or of mitomycin C markedly reduced the ability of phagocytosis. However, bactobolin, even in dose of 1 μg/ml, did not reduce the phagocytosis activity of macrophages. The treatment with 1 $\sim$ 0.01 μg/ml for 30 minutes did not reduce the number of adherent cells in each monolayer culture.

Inhibitory Effect of Bactobolin on Blastogenesis of Mouse Spleen Cells by Concanavalin A (Con A) or Lipopolysaccharide (LPS)

The inhibitory effect of bactobolin on blastogenesis of mouse spleen cells stimulated by Con A as T cell mitogen or LPS as B cell mitogen in cultures was studied. Each mitogen and bactobolin, adriamycin, mitomycin C or bleomycin were added to mouse spleen cell cultures at the start of culture.

As shown in Table 4, the ID_{50} of bactobolin was greater than that of adriamycin or mitomycin C and was similar to that of bleomycin. The inhibitory effect of these substances was the same for Con A and LPS treatments.

Table 4. The concentrations of antitumor substances inhibiting blastogenesis caused by mitogens.

	ID_{50}			
	Con A		LPS	
	μg/ml	Ratio	μg/ml	Ratio
Bactobolin	0.07	0.88	0.09	1.29
Mitomycin C	0.008	0.1	0.004	0.05
Adriamycin	0.02	0.25	0.009	0.13
Bleomycin	0.08	1.00	0.07	1.00

1×10^6 spleen cells/ml.
The radioactivity of ^{3}H-thymidine into spleen cells cultured for 3 days was 4780 c.p.m. in Con A-treated cultures, and was 3538 c.p.m. in LPS-treated cultures without any antitumor substance.

Inhibitory Effect of Bactobolin on the Production of Colony-forming-unit in Culture (CFU-C) of Murine Bone Marrow Progenitor Cells Stimulated with LPS-induced Colony-stimulating Factor (CSF)

To determine the effect of bactobolin on leucocytes, the influence of bactobolin on the production of CFU-C of mouse bone marrow cells was examined. Bactobolin and the other antitumor substances were added at the start of culture. The cultures contained CSF produced in CDF_1 mouse serum collected from mice after injection of LPS. As shown in Fig. 5, among the antitumor antibiotics tested, the inhibitory effect of bactobolin on the production of CFU-C was less than that of adriamycin or mitomycin C. ID_{50} value of these antibiotics were as follows: bactobolin, 0.034 μg/ml; bleomycin, 0.11 μg/ml; adriamycin, 0.0024 μg/ml; mitomycin C, $<$0.0001 μg/ml. The production of CFU-C of mouse bone marrow cells was most susceptible to mitomycin C and least susceptible to bleomycin. It would appear that bactobolin may not affect the differentiation or the derivation of bone marrow stem cells to granulocytes-macrophages.

Fig. 5. Inhibitory effects of antitumor substances on the production of CFU-C of mouse bone marrow cells stimulated by LPS-induced CSF.

BLM, bleomycin; BB, bactobolin; ADM, adriamycin; MMC, mitomycin C.

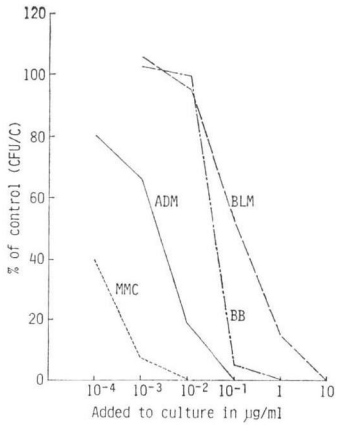
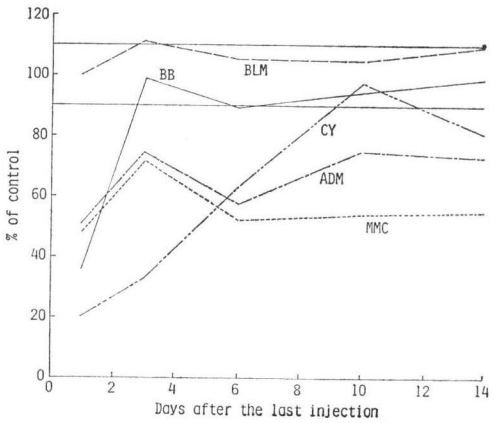


Fig. 6. The influence of bactobolin on the number of leucocytes in peripheral blood of mice.



The number of leucocytes in control was 10,108±1,268/mm³.

BLM, 10 mg/kg/day of bleomycin; BB, 2.5 mg/kg/day of bactobolin; ADM, 5 mg/kg/day of adriamycin; MMC, 2.5 mg/kg/day of mitomycin C; CY, 100 mg/kg/day of cyclophosphamide.

Standard deviation did not exceed 15%.

Leucocyte Count in Peripheral Blood from Mice Injected with Bactobolin or Other Antitumor Substances

It has been known that therapy by antitumor substances, which inhibit leukemia such as L-1210 also reduce the number of leucocytes in peripheral blood of animals[13].

In order to determine whether bactobolin reduced the number of leucocytes in peripheral blood of mice, mice were given 2.5 mg/kg/day of bactobolin daily for 5 days intraperitoneally and the number of leucocytes in peripheral venous blood was counted. Intraperitoneal injections of mitomycin C in 2.5 mg/kg/day, adriamycin in 5 mg/kg/day, cyclophosphamide in 100 mg/kg/day and bleomycin in 10 mg/kg/day were examined for comparison. As shown in Fig. 6, on 1 day following the last of 5 consecutive injections, all antitumor substances tested, except bleomycin, reduced the number of leucocytes at 3 days after the last injection, the number of leucocytes in mice injected with bactobolin was returned to the normal range, and thereafter fluctuated within the normal range. However, in the case of adriamycin, mitomycin C and cyclophosphamide recovery required a longer period. Bactobolin appears to reduce the number of leucocytes in peripheral blood for a short period and this reduction is reversible.

Discussion

Bactobolin has antibacterial activity in a broad spectrum and prolongs the survival period of L-1210-inoculated mice[1,2]. The administration of 5~0.15 mg/kg/day prolongs survival period in varied dose schedules. The most effective treatment schedule was daily administration of 0.6 mg/kg/day for 10 days.

The acute toxicity of bactobolin (LD_{50}) was estimated at 4.3 mg/kg in ICR mice[2] and 6.25~12.5 mg/kg in CDF_1 mice by a single intraperitoneal injection. However, mice tolerated the ad-

ministration of as much as 2.5 mg/kg/day for 10 days and 5 mg/kg/day every 2 days for 3 doses with a survival time of more than 16 days. The accumulation of bactobolin was shown to be very slight.

In experiments testing the effect of bactobolin on immune responses, it was shown that bactobolin was not an immunosuppressant on cell-mediated immune responses. The administration of bactobolin at 2~3 days after immunization, suppressed the increase of antibody-forming cells, but did not suppress at the time of immunization. This suggests that bactobolin may inhibit the differentiation of B cell to antibody-producing cells. Bactobolin was not effective on mouse T cell leukemia EL-4, suggesting that bactobolin may be effective against plasma cell tumors. Bactobolin appears to have low toxicity to lymphoid cells involved in host defense mechanisms.

Bactobolin is a unique antitumor antibiotic since it is effective on L-1210 mouse leukemia without suppressing cell-mediated immune response and the number of leucocytes. The experimental data suggest that bactobolin is worthy of clinical test.

Acknowledgements

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