ENHANCEMENT OF IMMUNE RESPONSES AND POSSIBLE INHIBITION OF SUPPRESSOR CELLS BY ACLACINOMYCIN A

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Antitumor antibiotics were examined as possible candidates that possess activity which inhibits preferentially suppressor cells in comparison with effector cells. In screening for such compounds among known antibiotics, aclacinomycin was found to augment antibody formation and delayed-type hypersensitivity in mice over a wide concentration range. The addition of aclacinomycin to mouse spleen cell cultures also enhanced antibody formation *in vitro*. The generation of suppressor cells or the suppressor activity *per se* in mice immunized with high doses of SRBC was reduced by aclacinomycin. These results suggest that the drug may possibly inhibit suppressor cells selectively. The administration of aclacinomycin at low doses exhibited antitumor effects on IMC carcinoma; the effect was not dose-dependent.

Aclacinomycin A is an anthracycline antitumor antibiotic[1,2] which has a significantly lower cardiotoxicity than adriamycin[3]. It has been reported to exhibit therapeutic effects on leukemia and lymphoma[3].

In this paper, we report the action of aclacinomycin in enhancing immune responses and its possible action in inhibiting suppressor cell activity.

Materials and Methods

Mice

CDF_1 mice (Balb/c×DBA/2, female, 8~10 weeks old) were obtained from the Institute of Medical Science, University of Tokyo and Shizuoka Laboratory Animal Agriculture Cooperative Association, Shizuoka, Japan, They were housed in plastic filtered top mouse cages and fed a diet of sterilized mouse pellets (FR-1, Funabashi Farm Co. Ltd., Chiba, Japan) and water *ad libitum*.

Aclacinomycin, other antitumor substances

Aclacinomycin A hydrochloride (ACM) was prepared according to the procedure reported previously[1]. Adriamycin HCl (ADM, Kyowa Hakko Co. Ltd., Tokyo, Japan), mitomycin C (MMC, Kyowa Hakko Co. Ltd., Tokyo, Japan), bleomycin (BLM, Nippon Kayaku Co. Ltd., Tokyo, Japan) and cyclophosphamide ("Endoxan", Shionogi & Co. Ltd., Osaka, Japan) were employed as controls. These compounds were dissolved in saline and injected intraperitoneally into mice (the desired doses being administered at 0.25 ml/mouse). For the *in vitro* experiments to test their effect on lymphocyte cultures these compounds were dissolved in medium and added to cultures at 0.05 ml/ml of medium.

Immune responses

Delayed-type hypersensitivity (DTH) to sheep red blood cells (SRBC) was tested according to the method described previously[4].

Antibody formation to SRBC was determined by a hemolytic plaque assay[5] using CUNNINGHAM's glass chamber[6]. Mice were immunized by intravenous injection of 10^8 SRBC, and 4 days thereafter, the direct plaque-forming cells (PFC) in the spleen of each mouse were enumerated. Five mice were employed in each group.

Antibody formation in spleen cell cultures was examined by MISHELL and DUTTON's method[7] as modified by CLICK *et al*[8].

The influence of aclacinomycin and the other antitumor antibiotics on the mitogenicity of lectins was examined as reported previously[9]. Mouse spleen cells, at 1×10^6 cells/ml, were cultured in a microplate (Falcon 3042, Div. Becton, Dickinson & Co., Oxnard, Calif.) at 37°C for 3 days in 5% CO_2 and air. The concentrations of concanavalin A (Con A, Pharmacia, Uppsala, Sweden), Lipopolysaccharide (LPS, Difco Laboratories, Detroit, Mich., U.S.A.) and Phytohemagglutinin P (PHA-P, E. Y. Laboratories, San Mateo, Calif., U.S.A.) in each culture were 0.5 μg/ml, 2 μg/ml and 5 μg/ml, respectively, unless otherwise noted. Triplicate cultures were performed in each assay.

Test of suppressor cell activity

Induction of suppressor cells by immunization with a large dose of SRBC was examined using the method described by WEITZMANN[10] and ANACLERIO *et al.*[11] for antibody formation. CDF_1 mice (5 mice in each group) were immunized with 1×10^9 or 5×10^9 SRBC intravenously and aclacinomycin was simultaneously injected intraperitoneally; 4 days later, the number of PFC was determined.

The transfer of suppressor cells induced by a large dose of SRBC into syngeneic hosts was performed as follows: CDF_1 mice (7 mice in each group) were immunized by intravenous injection of 5×10^9 SRBC; 2 days later they were given aclacinomycin A intraperitoneally, and 5 days after the immunization, the spleen cells were collected aseptically. The cells were washed with DULBECCO's modified phosphate buffered saline (DPBS) 3 times and administered intravenously to CDF_1 mice (5×10^7 spleen cells in 0.2 ml of DPBS per mouse). Three hours later, the mice were injected with 10^8 SRBC intravenously and 4 days thereafter, PFC were determined.

The suppressor cell activity in the DTH response to SRBC was tested by the method described by YAMAGUCHI and KISHIMOTO[12] and LAGRANGE *et al.*[13] CDF_1 mice (5 mice in each group) were immunized by the intravenous route with 10^8 SRBC for an optimum response or 10^8 SRBC for induction of suppressor cells and were simultaneously given aclacinomycin intraperitoneally or orally. After 4 days, the DTH response was elicited by subcutaneous injection of 10^8 SRBC to the footpad of the hind paw and 24 hours thereafter, the resulting edema was measured with a caliper.

Production of CFU-C in mouse bone marrow cell culture

Mouse bone marrow cells were cultured in soft agar medium with colony-stimulating factor (produced in mice by injection of LPS[15]) according to the method described previously[14].

Test of antitumor effect against IMC carcinoma

IMC carcinoma[4] was maintained in CDF_1 mice by serial intraperitoneal transplantation every 7 days. The cells were harvested and inoculated into the groin of CDF_1 mice subcutaneously (1×10^6 cells/mouse). Mice were given aclacinomycin daily for 5 days from day 1 or day 8 after inoculation of the tumor cells. Eight mice were used per group. Thirty days after inoculation, the tumors were extirpated and weighed.

Results

Influence of Aclacinomycin on Antibody Formation and Delayed-type Hypersensitivity to SRBC in Mice

Aclacinomycin, adriamycin, cyclophosphamide, mitomycin C or bleomycin was injected into mice daily for 4 days and one day thereafter, the mice were immunized by intravenous injection of 10^8 SRBC for antibody formation or by subcutaneous injection of 10^8 SRBC into the footpad of the hind paw for DTH. After four days antibody formation was examined by enumerating plaque-forming cells in the spleen and DTH was measured following injection to 10^8 SRBC into the other hind footpad. These substances were given at doses which showed antitumor activity.

The results are shown in Table 1. An increase in the number of plaque-forming cells was seen only with aclacinomycin (2 mg/kg daily); the other antitumor substances did not show any increase.

Table 1. Effect of antitumor substances on immune responses.

Antitumor substance[1] in mg/kg		On day 0	Antibody formation[2] PFC/spleen	DTH[3] Increase of footpad thickness (×0.1 mm)
—		10^8 SRBC	162,000±36,400	6.6±0.8
ACM	2.0	〃	311,000±19,100	11.2±2.9
	0.5	〃	153,900±14,000	8.1±1.2
ADM	1.0	〃	108,000±12,360	9.1±2.4
	0.25	〃	172,000±43,000	7.8±1.6
CY	100	〃	0	0.6±1.1
	25	〃	171,000±37,300	9.9±1.1
MMC	2.0	〃	66,420±12,300	0.6±1.6
	0.5	〃	176,580±23,170	9.8±1.3
BLM	5.0	〃	152,280±13,800	Not tested
	1.25	〃	147,420±10,500	〃

1) Mice were administered aclacinomycin (ACM), adriamycin (ADM), cyclophosphamide (CY), mitomycin C (MMC) or bleomycin (BLM) for 4 days; thereafter, they were immunized with 10^8 SRBC.

2) Four days after the immunization by intravenous injection of 10^8 SRBC, the number of plaque-forming cells (PFC) was enumerated by a hemolytic plaque assay.

3) Four days after the immunization by subcutaneous injection of 10^8 SRBC to the hind footpad, mice were injected with the same number of SRBC to the other hind paw. Twenty-four hours later, the resultant edema was measured.

By contrast, not only aclacinomycin but also adriamycin (1 mg/kg/day), cyclophosphamide (25 mg/kg/day) and mitomycin C (0.5 mg/kg/day) augmented the DTH. The doses of these compounds given were about 1/16~1/20 their LD_{50}. Of the compounds examined, aclacinomycin showed the strongest enhancement of DTH. The data indicate that aclacinomycin, administered at doses effective against L-1210 and other murine tumors, can augment IgM antibody formation and DTH response to SRBC suggesting that the mouse immune system is activated by aclacinomycin.

The influence of aclacinomycin and the other antitumor compounds on the spleen and thymus was examined by measuring the weight of these organs one day after the mice were injected intraperitoneally with these drugs for 4 successive days. Aclacinomycin (2 mg/kg/day, about 1/16 the LD_{50}) reduced the weight of the spleen by about 30% whereas the weight of the thymus was not affected. Adriamyicn showed almost the same effect as aclacinomycin. Mitomycin C reduced the weight of the spleen more markedly than that of the thymus and cyclophosphamide preferentially affected the thymus. In all cases, however, the number of nucleated spleen cells per weight (mg) was not changed: the number of cells/mg was $1.6 \sim 1.8 \times 10^6$. Thus, the results indicate that the reduction in weight of the spleen was due to the decrease in the number of nucleated cells.

We also examined the effect of aclacinomycin administration at the time of immunization on antibody formation and DTH. Mice were immunized by intravenous injection of 10^8 SRBC and, at the same time, aclacinomycin was given intraperitoneally at various doses. As shown in Table 2, aclacinomycin augmented antibody formation and DTH. Even a dose as small as 0.1~1.0 μg/mouse (which does not inhibit tumor growth) exhibited this effect.

Aclacinomycin is well absorbed by oral administration[3]. The stimulatory effect of aclacinomycin on DTH response was also observed when doses of 0.1 to 100 μg/mouse were given orally (data not shown).

Table 2. Effect of aclacinomycin on immune responses in mice.

Immunization		Antibody formation[1] PFC/spleen	DTH[2] Increase of footpad thickness (×0.1 mm)
10^8 SRBC		486,400± 27,000	6.8±0.8
10^8 SRBC	100 μg/mouse, i.p.	916,000±108,800	8.3±1.7
〃	10 μg	864,000±152,600	14.5±1.4
〃	1 μg	816,000±152,600	10.6±1.4
〃	0.1 μg	784,000± 51,400	9.6±1.4

1) Mice were immunized with 10^8 SRBC and were given aclacinomycin intraperitoneally. Four days later, antibody formation was determined by a hemolytic plaque assay.

2) Mice were immunized with 10^8 SRBC by subcutaneous injection in the hind footpad and given aclacinomycin intraperitoneally; four days later, the mice were injected with the same number of SRBC to the other hind footpad. The resultant edema was measured 24 hours later.

Effect of Aclacinomycin on Antibody Formation *In Vitro*

The effect of aclacinomycin on antibody formation in dissociated spleen cell cultures was tested. As seen in Table 3, the addition of aclacinomycin at 0.001~0.01 μg/ml at the start of the incubation resulted in a 2-fold increase in the number of plaque-forming cells. At 0.1 μg/ml, the number of viable cells recovered was reduced markedly and antibody formation was not detected. In comparison with aclacinomycin, the addition of adriamycin at 0.001~1 μg/ml failed to show any stimulatory effect on antibody formation. Adriamycin at 0.1 μg/ml, reduced the number of viable cells only slightly; moreover, antibody forming cells were detected at this concentration of drug.

In order to determine the optimum time for the addition of aclacinomycin during the course of culture, 0.01 μg/ml of aclacinomycin or adriamycin was added at 0, 1, 2 or 3 days after the start of the

Table 3. Effect of aclacinomycin on antibody formation *in vitro*.

Addition to cultures[1] (μg)		No. of viable cells ×10^6 cells/ml[2]	PFC/ culture[3]
10^6 SRBC	—	3.5	1,410
〃	ACM 0.1	1.6	0
〃	〃 0.01	3.5	2,850
〃	〃 0.001	3.7	2,100
〃	〃 0.0001	3.8	1,440
〃	ADM 1	0.7	0
〃	〃 0.1	2.8	330
〃	〃 0.01	3.4	960
〃	〃 0.001	3.2	1,560

1) Spleen cell cultures were prepared by the method of MISHELL and DUTTON and aclacinomycin (ACM) or adriamycin (ADM) was added to cultures. After 4 days, antibody formation was determined by a hemolytic plaque assay.

2) Standard deviation did not exceed 5 %.

3) Standard deviation did not exceed 10 %.

Table 4. Effect of addition of aclacinomycin and adriamycin at different times during course of culture on antibody formation *in vitro*.

Addition to cultures[1]		No. of viable cells ×10^6 cells/ml[2]	PFC/ culture[3]
10^6 SRBC	—	5.3	1,170
〃	ACM, day 0	5.5	3,360
〃	〃 〃 1	4.8	3,000
〃	〃 〃 2	4.8	1,620
〃	〃 〃 3	4.0	2,070
〃	ADM, day 0	4.6	990
〃	〃 〃 1	4.6	1,470
〃	〃 〃 2	5.3	1,050
〃	〃 〃 3	4.8	1,020

1) Each culture was added 0.01 μg/ml of aclacinomycin (ACM) or adriamycin (ADM) at different times during the course of culture. Four days after the start of the incubation, antibody formation was determined.

2) Standard deviation did not exceed 8 %.

3) Standard deviation did not exceed 10 %.

incubation. Table 4 reveals that the maximum effect of aclacinomycin was observed when it was added after 0 or 1 day. Addition of aclacinomycin at the onset of the incubation resulted in the maximum stimulation suggesting that the agent may activate chiefly macrophages and/or helper T cells[16,17]. In contrast, the addition of adriamycin at any of the times tested did not provide any stimulatory effect.

Inhibitory Effect of Aclacinomycin on Blastogenesis of Spleen Cells Induced by Mitogens

The influence of aclacinomycin, adriamycin and mitomycin C on blast transformation of murine splenocytes was examined. Spleen cells were cultured with Con A, PHA-P or LPS and one of the antibiotics; after 3 days incubation the rate of incorporation of ^{3}H-thymidine into the spleen cells was determined. As shown in Table 5, aclacinomycin was the most effective agent used to suppress blast transformation induced by the mitogens. In comparison with aclacinomycin on a μg basis, the IC_{50} for adriamycin was about 5-fold greater using Con A and about 3~4 times higher with PHA and LPS as mitogens. In addition, mitomycin C was required at 2~4 times greater concentration with these mitogens.

Inhibitory Effect of Aclacinomycin on Production of CFU-C of Mouse Bone Marrow Cells

Since aclacinomycin was more effective than adriamycin or mitomycin C in suppressing blast transformation of spleen cells induced by mitogens, we examined the inhibitory effect of aclacinomycin, adriamycin and mitomycin upon the production of CFU-C by mouse bone marrow cells *in vitro*. As shown in Table 6, aclacinomycin was far less inhibitory than adriamycin or mitomycin; the IC_{50} of aclacinomycin was approximately 3-fold greater than that of adriamycin. The results reveal that aclacinomycin exhibits a lower cytotoxic effect upon cultured mouse bone marrow cells than the other drugs, although aclacinomycin has the strongest inhibitory effect against blast transformation of spleen cells.

Table 5. Relative inhibitory concentrations (IC_{50}) of aclacinomycin, adriamycin and mitomycin C on blast transformation of mouse spleen cells stimulated by mitogens.[1)]

Mitogens	IC_{50} in μg/ml		
	Aclacinomycin	Adriamycin	Mitomycin C
Con A	0.039	0.22	0.098
PHA-P	0.037	0.13	0.074
LPS	0.022	0.088	0.092

1) Mouse spleen cells were cultured with 0.5 μg/ml of Con A, 5 μg/ml of PHA-P or 2 μg/ml of LPS and various concentrations of each antibiotic for 3 days at 37°C in 5% CO_2. ^{3}H-Thymidine (1 μCi/ml) was added 18 hours before the assay and incorporation into cultured cells was determined. The c.p.m. of spleen cells/culture stimulated with each mitogen was as follows: spleen cells without mitogens, 1,153±102; Con A, 18,200±756; PHA-P, 6,640±648; LPS, 9,075±222.

Table 6. Relative inhibitory concentrations (IC_{50}) of aclacinomycin, adriamycin and mitomycin C on production of CFU-C by mouse bone marrow cells.[1)]

Antibiotics	IC_{50} in ng/ml
Aclacinomycin	6.8
Adriamycin	2.4
Mitomycin C	<0.1

1) Mouse bone marrow cells were collected from the femora and suspended in 0.3% agar containing alpha MEM supplemented with 10% fetal calf serum, 5% horse serum and colony-stimulating factor at 7.5×10^4 cells/ml. The cells were cultured with antibiotics; 8 days later, macrophage-granulocyte colonies (CFU-C) were counted.

The observations described above suggest that among lymphoid cell populations some cells are affected and/or activated by aclacinomycin with a resultant enhancement of the immune response. It is conceivable that one of the possible mechanisms of action of aclacinomycin with respect to the immune responses may be a selective inhibition of the formation of suppressor cells without a concomitant inhibition of the generation of immunocompetent effector cells. This was examined in the following experiments.

Inhibition of Suppressor Cell Activities by Aclacinomycin

It has been observed that a large dose of antigen generates suppressor cells and reduces the immune responses seen with optimal doses of an immunogen[10~12]. The effect of aclacinomycin A on the formation of suppressor cells in antibody formation and DTH against SRBC was tested in mice immunized with a large dose of antigen.

Table 7. Reduction of suppressor activity by aclacinomycin on antibody formation.[1]

Immunization	Treatment	PFC/spleen	% of control
I 1×10^8 SRBC i.v.	None	191,200± 4,100	
1×10^9 SRBC i.v.	None	92,000±18,400	48
1×10^9 SRBC i.v.	ACM 100 μg i.p.	297,300±26,400	156
〃	〃 〃 p.o.	130,410±11,300	68
II 1×10^8 SRBC i.v.	None	177,400±14,800	
5×10^9 SRBC i.v.	None	61,300±13,400	35
5×10^9 SRBC i.v.	ACM 25 μg i.p.	162,700± 8,300	92
〃	ADM 80 μg i.p.	62,700± 2,000	35
〃	〃 20 μg 〃	69,300± 7,800	39

[1] Mice were immunized with 10^8, 10^9 or 5×10^9 SRBC i.v. and were given the appropriate dose of aclacinomycin (ACM) i.p. or p.o. or adriamycin (ADM) i.p. Four days thereafter, the number of plaque forming cells in spleens was enumerated.

Table 8. Effect of aclacinomycin on suppressor activity.[1]

Mice transferred	Treatment	PFC/spleen	% of control
Normal spleen cells	None	51,744±2,254	
Suppressor	None	7,448±1,640*	14
Suppressor	ACM 100 μg i.p.	35,280±3,368**	68
〃	〃 10 μg 〃	23,520±1,598**	45
〃	〃 1 μg 〃	17,400±1,737***	34
Suppressor	ADM 80 μg i.p.	10,780±2,665†	21
〃	〃 8 μg 〃	8,624±1,294†	17

[1] CDF_1 mice were immunized with 5×10^9 SRBC i.v.; 2 days later, they were injected with aclacinomycin (ACM) or adriamycin (ADM) i.p. and 5 days after the immunization, spleen cells of those mice (suppressor) were transferred to syngeneic mice and 3 hours later, were immunized with 10^8 SRBC i.v. Four days after the transfer, antibody formation was determined by counting PFC.

* $P<0.01$ *vs.* control.

** $P<0.01$ *vs.* suppressor.

*** $P<0.05$ *vs.* suppressor.

† Not significant.

Mice were immunized by intravenous injection of 10^8 or 10^9 SRBC and after 4 days, the number of PFC was determined. As shown in Table 7, the number of PFC was reduced by about 50~65% in the case of the mice immunized with 1×10^9 or 5×10^9 SRBC; however, the administration of 25 to 100 μg of aclacinomycin at the time of immunization enhanced the production of PFC. The dose of aclacinomycin employed was about 1/6 to 1/25 LD_{50}. Adriamycin, in the same dose range, did not alter the repressed state.

In order to determine whether the enhancement of antibody formation is due to an inhibition of suppressor cell activity by aclacinomycin, it was tested by the method of ANACLERIO *et al.*[11] Mice were immunized with a large dose of antigen (5×10^9 SRBC) to induce suppressor cell activity in their spleen cells (group A). A second group of mice received the same treatment, however aclacinomycin was given on day 2 after the immunization (group B). Then, the spleen from each group were transferred to syngeneic mice on day 5 after the immunization and antibody formation was determined.

As shown in Table 8, the transfer of the spleen cells (group A) resulted in a marked reduction of antibody formation; by contrast the transfer of spleen cells from group B reduced it only slightly.

These findings indicate that aclacinomycin may inhibit the generation of suppressor cells induced by immunization with a large dose of antigen. Therefore, the results shown in Tables 7 and 8 suggest that aclacinomycin affects suppressor cells selectivley during antibody formation.

Similar results were obtained with aclacinomycin when the DTH response was examined. Intravenous injection of 10^5 SRBC is the optimum dose for establishment of DTH[11]; if 10^8 SRBC were used, the DTH response was markedly reduced by generation of T suppressor cells[12]. As shown in Table 9, the DTH response in mice given aclacinomycin A was equal to or greater than that in the control mice.

Antitumor Effect of a Low Dose of Aclacinomycin against IMC Carcinoma

Aclacinomycin, at doses (1 μg to 10 μg/mouse) lower than those exhibiting antitumor activities against murine transplantable tumors[3], effectively augmented the immune responses. Therefore,

Table 9. Reduction of suppressor activity by aclacinomycin on delayed-type hypersensitivity.[1]

Immunization	Treatment	Increase of footpad thickness (×0.1 mm)	% of control
I 10^5 SRBC i.v.	None	7.1±1.5	100
10^8 SRBC i.v.	None	2.2±1.2	31
10^8 SRBC i.v.	ACM 100 μg i.p.	5.4±2.1	76
〃 〃	〃 〃 p.o.	5.3±1.1	75
II 10^5 SRBC i.v.	None	5.4±1.8	100
10^8 SRBC i.v.	None	2.1±1.5	39
10^8 SRBC i.v.	ACM 100 μg p.o.	7.1±1.7	132
〃 〃	〃 10 μg 〃	9.2±1.1	170
〃 〃	〃 1 μg 〃	7.4±2.0	137
10^8 SRBC i.v.	ACM 100 μg i.p.	5.2±1.3	96
〃 〃	〃 10 μg 〃	7.4±2.2	137
〃 〃	〃 1 μg 〃	5.8±1.4	107

1) CDF_1 mice were immunized with 10^5 or 10^8 SRBC i.v. and were given aclacinomycin (ACM) i.p. or p.o.; four days later, 10^8 SRBC were injected s.c. to the hind footpad. The resultant edema was measured 24 hours later.

Table 10. Antitumor effect of aclacinomycin against IMC carcinoma in CDF_1 mice.[1]

Aclacinomycin in μg/mouse	Therapy			
	Days 1~5		Days 8~12	
	Tumor w.t. (mg)	% Inhibition	Tumor w.t. (mg)	% Inhibition
10	2,670±924	49.6	2,523± 890	52.4
1	1,820±553	65.6	2,616± 497	50.6
0.1	1,913±649	63.9	2,495±1,433	52.9
0	5,295±733			

[1] Mice were implanted with 1×10^6 IMC carcinoma cells into the groin subcutaneously and were given aclacinomycin daily for 5 consecutive days orally from day 1 or 8 after implantation; after 30 days tumors were extirpated and weighed.

the effect of aclacinomycin at lower doses was examined against a syngeneic tumor, IMC carcinoma. As shown in Table 10, oral administration of aclacinomycin at 0.1 μg, 1 μg or 10 μg/mouse daily for 5 days was effective in suppressing growth of the tumor by about 50%~60% when given at the time or subsequent to the implantation of the tumor.

Discussion

The administration of aclacinomycin prior to immunization enhanced antibody formation and the establishment of DTH to SRBC. This observation indicates that aclacinomycin does not affect lymphoid cells involved in these immune responses. In comparison to other antitumor substances in the same dose range (estimated from the LD_{50}) only aclacinomycin augmented both immune responses. Moreover, the enhancing effect of aclacinomycin on the immune responses was observed when the drug was administered at the time of immunization. Both immune responses were augmented by single injections of 0.1 to 100 μg/mouse of aclacinomycin. Since it was shown that aclacinomycin is effective in prolonging the survival of tumor-bearing mice at 0.5~5 mg/kg[3] (estimated to be about 10~100 μg/mouse), these results suggest that immunocompetent cells and other lymphoid cells involved in these immune responses are not killed by aclacinomycin but rather that their activities may be stimulated.

The effect of aclacinomycin upon antibody formation *in vivo* was also observed in *in vitro* experiments. Thus, the addition of 0.01~0.001 μg of aclacinomycin/ml at the start of the culture increased the number of plaque-forming cells (Table 3). On the other hand, the addition of adriamycin at 0.001~1 μg/ml exhibited no effect. The optimum time for the addition of aclacinomycin to augment antibody formation *in vitro* was determined to be at the initiation of the culture. This suggests that within the lymphoid cell population antigen-presenting cells such as macrophages, and/or helper T cells might be activated by aclacinomycin[16,17].

In comparison with adriamycin and mitomycin C, aclacinomycin exhibited the strongest inhibitory effect on blast transformation of spleen cells stimulated with mitogens. However, aclacinomycin did not show a stronger inhibitory effect of CFU-C production by mouse bone marrow cells. This result strongly suggested that aclacinomycin affected blast transformation of only one or some types of cells in the lymphoid cell population.

Although aclacinomycin showed a strong inhibitory effect on blast transformation, it did not influence antibody formation or the DTH response. These observation suggested that aclacinomycin may inhibit suppressor cell activity. This conclusion was supported by the observation that aclacinomycin partially reversed the reduction of immune responses caused by generation of suppressor cells in two different systems. It has been reported that the anthracycline antitumor antibiotics, adriamycin and daunomycin at high concentrations (1/2~1/4 LD_{50}) can reduce suppressor activity induced by high

doses of antigen[11]. In the present study however, lower doses of aclacinomycin (about 1/25 LD_{50}) were required to reduce suppressor activity. Thus, suppressor cells appear to be more sensitive to aclacinomycin than to adriamycin.

Recently, ORBACH-ARBOUYS *et al.*[18] found that the treatment of irradiated or thymectomized mice with aclacinomycin prior to immunization augmented antibody formation and cell-mediated immune responses. They concluded that the effect of aclacinomycin was due to its effect on T cells. As shown in the present results, the inhibition of suppressor cell activity may be the basis for the enhancement of the immune responses observed with aclacinomycin.

The effect of aclacinomycin against IMC carcinoma at doses which are not effective for the inhibition of L-1210 was found to be independent of the dose used. This finding suggested that the antitumor effect may be due to the enhancement of immune responses in tumor-bearing hosts by aclacinomycin[4].

The experimental data presented in this paper suggests that one of the possible mechanisms of activity of aclacinomycin with respect to the enhancement of immune responses may involve a reduction of suppressor cell activity.

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