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Production of Antitumor T-Cells in Tumor-bearing Mice Treated with Tumor Vaccine and 6-Mercaptopurine[1]

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ABSTRACT

Treatment with both L1210 murine leukemia cell vaccine (L1210 vaccine) and 6-mercaptopurine (6-MP) induced antitumor effector cells in the spleen and peritoneal cavity of L1210-bearing mice. The *in vivo* neutralization test showed that the spleen cells and peritoneal cells of mice treated with both agents, but not with either agent alone, prolonged the life span of animals simultaneously inoculated i.p. with live L1210 cells. These results indicate that these antitumor cells were associated with the augmented therapeutic response in L1210-bearing mice treated with both agents.

The neutralizing activity of peritoneal cells was located to a fraction not adhering to plastic flasks and abolished by the treatment of anti-Thy 1.2 antibody and complement, indicating that they were T-cells. The *in vitro* antiproliferation test confirmed these observations. The spleen cells and peritoneal T-cells of these mice suppressed L1210 proliferation. Their activity was tumor specific since they suppressed the *in vitro* proliferation of L1210 but not P388 and L5178Y cells. The *in vivo* association of antitumor T-cells with the augmented therapeutic effect was substantiated by the finding that rabbit anti-mouse thymocyte globulin abolished the induced therapeutic effect.

INTRODUCTION

Most chemotherapeutic agents suppress host antitumor immunity (1). However, some of them augmented host antitumor immunity under specific experimental conditions. It was noted that the augmentation was achieved by agents directly interacting with DNA molecules such as cyclophosphamide (5, 6, 14, 16, 17, 20), busulfan (19), Adriamycin (3, 15, 17, 20), daunomycin (13), mitomycin C (13), and one of nitrosourea derivatives (13). Since all these agents are supposed to kill host cells without selectivity, they exerted the augmenting effect under limited experimental conditions specified by species and strains of animals and tumor species. In this regard, the antimetabolic agents exerting their anticellular effect in association with cell physiology may be considered as an alternative. In fact, under the limited experimental conditions, 6-MP[3] has long been known to augment the humoral and cell-mediated immune responses (2, 18). However, in antitumor immunity, it is not clarified that any of antimetabolic chemotherapeutic agents including 6-MP augments the host antitumor immunity in immunoprophylactic and immunotherapeutic conditions.

Recently, we found that, in combination, 6-MP, an antimetabolic agent, and L1210 murine leukemia cell vaccine produced an augmented therapeutic effect in L1210-bearing mice (8). A series of *in vivo* studies showed that 6-MP-induced reduction of tumor burden was only partially responsible for the augmented therapeutic response. Further study showed that the augmented therapeutic response was dependent on tumor cell vaccine as evidenced by the findings that it was achieved by the immunogenic (Con A-bound), but not by less immunogenic (Con A-free) vaccine and that it was tumor specific. These results suggested that the antitumor immunity induced in tumor-bearing mice was associated with the augmented therapeutic response. The present study is to substantiate this hypothesis and to show that 6-MP augmented host T-cell-mediated antitumor immunity.

MATERIALS AND METHODS

Cells and Animals. L1210 and P388 murine leukemia cells supplied by the National Cancer Institute (Bethesda, MD) were collected from the ascites of male syngeneic DBA/2Cr mice. L5178Y murine lymphoma cells were kindly supplied by Dr. Yahara and were made i.p. transplantable in BALB/c × DBA/2Cr F_1 (hereafter called $CD2F_1$) mice. Male $CD2F_1$ mice, histocompatible with DBA/2Cr mice, were used in the experiments. They were obtained from Charles River Japan, Inc. (Kanagawa, Japan).

Vaccination. Con A-bound L1210 vaccine was prepared as reported previously (12). Briefly, L1210 cells were incubated first for 1 h at 37°C with mitomycin C (200 µg/ml; Kyowa Hakko Co., Ltd., Tokyo, Japan) and then for 30 min on ice with Con A (165 µg/ml; Miles Laboratories, Kankakee, IL). After one washing, they were inoculated i.p. In experiments using Con A-free vaccine, the cells were incubated with mitomycin C but not with Con A.

Immunocytes. Single-cell suspensions of spleen and peritoneal cavity were prepared as reported (11), except that the spleens were either squeezed manually in a sterilized cellulose tube (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) for the *in vitro* test or homogenized in a rubber-coated glass homogenizer for the i.p. transfer test. Fractions of cells not adhering to plastic flasks were prepared as reported (11). In L5178Y, mice sensitized with 10^7Con A-bound L5178Y vaccine and pyran copolymer according to the protocol used in L1210 (11) were inoculated i.p. with 10^3 live L5178Y cells. About 2 weeks later, they were reinoculated i.p. with 10^3 live L5178Y cells, and the peritoneal cells of surviving mice were used as a source of anti-L5178Y immunocytes. In Table 1, peritoneal cells of mice inoculated i.p. with 1 ml of 12% sodium caseinate in 0.85% sodium chloride solution (Difco Laboratories, Detroit, MI) 2 days before were also used as a source of effector cells.

Antitumor Activity Assay of Immunocytes. For this, we used the *in vivo* neutralization and *in vitro* antiproliferation tests as reported previously (11). In the *in vitro* antiproliferation test, spleen or peritoneal cells (10^6/ml) were incubated at 37°C with 10^3 tumor cells/ml in a CO_2 incubator (Hirasawa Works, Tokyo, Japan) (Stage 1). After 3 days, 50-µl aliquots of the mixture containing surviving tumor cells were removed and mixed with 1 ml RPMI 1640 (Grand Island Biological Co., Grand Island, NY) containing 10% fetal bovine serum (Grand Island Biological

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[3] The abbreviations used are: 6-MP, 6-mercaptopurine; Con A, concanavalin A.

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Co.,) 20 μM mercaptoethanol (Wako Pure Chemical Industries, Ltd., Tokyo), and kanamycin (100 μg/ml; Banyu Pharmaceutical Co., Ltd., Tokyo, Japan). After a further 3-day incubation, tumor cell concentrations were determined on a Model Z_B Coulter Counter (Coulter Electronics, Inc., Hialeah, FL) (Stage 2). With immunocytes of L5178Y-sensitized mice, 10% fetal bovine serum was substituted for by 2% normal $CD2F_1$ male mouse serum in Stage 1.

Treatment of Immunocytes with Anti-Thy 1.2 Antibody and Complement. Immunocytes (10^7/ml) were incubated for 1 h on ice in the presence or absence of monoclonal anti-Thy 1.2 antibody (1:2,000 to 1:7,000; Olac 1976, Ltd., Oxon, England). They were then centrifuged and incubated at 37°C for 30 min in the presence of rabbit Low-Tox-M complement (Cedarlane Laboratories, Ltd., London, Ontario, Canada) diluted to 1:10 to 1:20. The trypan blue exclusion test showed that, under these experimental conditions, about 90% of $CD2F_1$ mouse thymocytes were lysed (9). Throughout this treatment, cytotoxicity medium (Cedarlane) was used as the incubation and diluting medium.

Other Agents. 6-MP (Sigma Chemical Co., St. Louis, MO), normal rabbit globulin, and rabbit anti-mouse thymocyte globulin (Microbiological Associates, Walkersville, MD) were used. The globulins were reconstituted by adding distilled water as suggested by the supplier; 0.25-ml quantities were injected i.p. into the mice.

Evaluation of the Results. The results were analyzed for significance using the t test for the *in vitro* antiproliferation test and, for life span prolongation in multiple experiments with small numbers of mice, the paired t test was used (Tables 1 and 3). The survival of mice in experiments using more than 6 mice/group was analyzed by the Mann-Whitney test. The *in vivo* experiments were terminated at Day 45. Under the present experimental conditions, 45-day survivors free of tumor signs (*i.e.*, increased ascitic fluid and/or delayed palpable s.c. tumor growth) did not develop leukemia later and were considered cured.

RESULTS

Antitumor Cells in L1210-bearing Mice Treated with 6-MP and L1210 Vaccine. L1210-bearing mice were treated with L1210 vaccine and/or 6-MP, and the antitumor activity of their spleen cells and peritoneal cells was examined by the tumor neutralization test in the peritoneal cavity of recipient mice. Because donors treated with L1210 vaccine alone died within 15 days of the inoculation or, alternatively, their immunocytes were heavily contaminated with L1210 cells, the activity of cells from these animals was not assayed.

As shown in Table 1, the i.p. injection of spleen cells from L1210-bearing donors treated with 6-MP alone shortened the life span of the recipient mice. This adverse effect is ascribable to L1210 cell contamination of the spleen; the administration of 6-MP alone to L1210-bearing donors was not curative. In contrast, the transfer of spleen cells from L1210-bearing donors treated with both 6-MP and L1210 vaccine prolonged the life span of the recipients, although marginally, indicating that these spleen cells were antitumor effector cells.

Similar observations were made when peritoneal cells were transferred. Cells from L1210-bearing donors treated with 6-MP alone resulted in a shortening of the recipients' life span, whereas the transfer of peritoneal cells from donors treated with both 6-MP and L1210 vaccine prolonged the life span of the recipients (Table 1). These results suggest that 6-MP augmented the production of antitumor immunocytes by L1210 vaccine, leading to an augmentation of the therapeutic effect. Peritoneal cells (5 to 7 $\times$ 10^6) from donors treated with both agents were more effective than (Experiments 1 and 3) or as effective as (Experiments 2 and 4) their spleen cells (5 to 6 $\times$ 10^7) in prolonging the life span of L1210-bearing recipients.

Characterization of Antitumor Cells. We characterized the antitumor effector cells by testing their *in vitro* antiproliferation activity against L1210 cells. As shown in Table 2, spleen cells from L1210-bearing donors treated with 6-MP and vaccine, but not with 6-MP alone, suppressed the *in vitro* proliferation of L1210 cells. When donor spleen cells were treated with anti-Thy 1.2 antibody plus complement but not with complement alone, the suppression of L1210 cell proliferation was completely abolished. This finding indicates the splenic effector cells to be T-cells.

Peritoneal cells from L1210-bearing donors treated with 6-MP and L1210 vaccine were fractionated according to their adherence to plastic flasks. Nonadherent populations treated with complement alone suppressed L1210 proliferation to a greater degree than did those treated with anti-Thy 1.2 plus complement,

Table 1

In vivo antitumor activity of spleen- and peritoneal cells from L1210-bearing mice treated with 6-MP and L1210 vaccine

Mice inoculated i.p. with 10^2 live L1210 cells (Day 0) were given i.p. injections of 10^7 L1210 vaccine on Days 1 and 8, and/or with 6-MP (100 mg/kg) on Day 5. On Day 15 or 16, their unfractionated spleen cells (5 to 6 $\times$ 10^7) and peritoneal cells (5 to 7 $\times$ 10^6) were transferred i.p. to recipients simultaneously inoculated i.p. with 10^2 live L1210 cells. For controls, the equivalent numbers of immunocytes of sodium caseinate-inoculated mice not bearing L1210 were also assayed. The prolongation of survival time of recipients of "6-MP + vaccine" spleen and peritoneal cells was statistically supported at $P < 0.05$ by the paired t test as compared with the recipients of "6-MP" immunocytes or the controls (no immunocyte transfer).

	Survival of recipients							
	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
Transferred immunocytes	No. of cured mice/total	Survival days	No. of cured mice/total	Survival days	No. of cured mice/total	Survival days	No. of cured mice/total	Survival days
None	0/5	12 ± 0[a]	0/5	10 ± 0	0/10	11 ± 0	0/10	12 ± 0
Spleen cells of donors treated with								
6-MP	0/7	10 ± 0	0/6	9 ± 0				
6-MP + vaccine	0/7	15 ± 3	0/6	16 ± 4	0/6	13 ± 1	4/10	20 ± 2
Sodium caseinate[b]							0/10	11 ± 0
Peritoneal cells of donors treated with								
6-MP	0/6	9 ± 0	0/5	7 ± 0				
6-MP + vaccine	2/6	22 ± 6	0/8	14 ± 2	0/6	18 ± 6	5/10	19 ± 1
Sodium caseinate[b]							0/10	11 ± 0

[a] Mean ± SD.
[b] Donor mice were naive mice not inoculated with L1210 but inoculated with sodium caseinate by the protocol indicated in "Materials and Methods."

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Table 2

In vitro antiproliferation activity of spleen T-cells and peritoneal T-cells from L1210-bearing mice treated with 6-MP and L1210 vaccine

Spleen and peritoneal cells were prepared according to the protocol of Table 1 except for the doses of 6-MP (100 to 200 mg/kg). Day 11 spleen cells and Day 15 peritoneal cells not adhering to plastic flasks were treated with anti-Thy 1.2 antibody and/or complement. These immunocytes (10^6/ml) were assayed for antiproliferation of L1210 cells (10^3/ml in spleen cell experiments and 2×10^3 to 10^4/ml in peritoneal cell experiments). For further details, see "Materials and Methods."

	Immunocyte donors treated with			
	L1210 vaccine	6-MP	*In vitro* treatment of immunocytes	% of control L1210 cell concentration in the presence of immunocytes
Spleen cells				
Experiment 1	−	+	None	118 ± 4.3[a,b]
	+	+	None	69.0 ± 12[b]
Experiment 2	+	+	Complement	68.8 ± 4.1[c]
	+	+	Anti-Thy 1.2 antibody + complement	99.3 ± 11.2[c]
Peritoneal cells				
Experiment 1	+	+	Complement	66.8 ± 5.0[d]
	+	+	Anti-Thy 1.2 antibody + complement	80.4 ± 2.9[d]
Experiment 2	+	+	Complement	71.2 ± 3.8[e]
	+	+	Anti-Thy 1.2 antibody + complement	100 ± 11.8[e]

[a] Mean ± SD of quadruplicate determinations.
[b,c,d,e] Statistically significant at $P \leq 0.05$ by t test, respectively.

Table 3

In vivo antitumor activity of peritoneal T-cells from L1210-bearing mice treated with 6-MP and L1210 vaccine

Peritoneal cells (Day 15 in Experiments 1 and 2; Day 12 in Experiments 3 and 4) were prepared according to the protocol of Table 1 except for doses of 6-MP (150 mg/kg). These cells, unfractionated (Experiments 1, 2, and 4) or populations not adhering to a plastic flask, were untreated or were treated with anti-Thy 1.2 antibody and/or complement. These immunocytes (5×10^6/mouse in Experiments 1 and 2, 2×10^6/mouse in Experiment 3; 10^7/mouse in Experiment 4) were transferred i.p. to recipients simultaneously inoculated i.p. with live L1210 cells (10^2 in Experiments 1 and 4; 2×10^2 in Experiments 2 and 3). The prolongation of survival time of the complement group was statistically supported at $P < 0.05$ by the paired t test as compared with the anti-Thy 1.2 antibody + complement-treated and control groups.

	Survival[a] of recipients of peritoneal cells and live L1210 cells (days)			
Transferred peritoneal cells treated with	Experiment 1	Experiment 2	Experiment 3	Experiment 4
None	15 ± 0[b] (1/6)[c]			
Complement	15 ± 3 (0/7)	14 ± 1 (0/8)	14 ± 3 (0/8)	16 ± 0 (3/6)
Anti-Thy 1.2 antibody + complement	10 ± 0 (0/6)	12 ± 0 (0/8)	10 ± 0 (0/8)	11 ± 0 (0/6)

[a] Survival days of L1210-bearing mice. Survival of control mice was 11 ± 0 (Experiment 1), 12 ± 0 (Experiment 2), 12 ± 0 (Experiment 3), and 12 ± 0 (Experiment 4) days, respectively.
[b] Mean ± SD.
[c] Numbers in parentheses, number of cured mice/total.

again suggesting that the peritoneal effector cells were T-cells.

In some experiments, however, the treatment with anti-Thy 1.2 antibody plus complement abolished the antitumor activity of peritoneal cells incompletely (Table 2, Experiment 1). Thus, we tested by the *in vivo* neutralization test whether these antitumor peritoneal T-cells were effective *in vivo* (Table 3). Peritoneal cells of L1210-bearing mice treated with 6-MP and vaccine, nonfractionated (Table 3, Experiments 1, 2, and 4) or nonadherent population (Table 3, Experiment 3), were treated *in vitro* with complement or anti-Thy 1.2 plus complement or not treated and were examined for neutralizing activity. Table 3 showed that peritoneal cells treated with anti-Thy 1.2 antibody plus complement but not with complement alone abolished the antitumor activity, indicating that peritoneal T-cells were involved in the *in vivo* antitumor immunity in L1210-bearing mice treated with 6-MP and the vaccine.

Tumor Selectivity of Antitumor Cells. We examined tumor selectivity of peritoneal antitumor cells by comparing their antiproliferation effect on L1210, L5178Y, and P388 cells. As shown in Table 4, nonadherent populations of peritoneal cells sensitized with L1210 suppressed L1210 but not L5178Y and P388 proliferation, indicating the selective suppression of L1210 cell proliferation by the peritoneal effector cells. This conclusion was further substantiated by the finding that the peritoneal cells sensitized with L5178Y suppressed L5178Y but not L1210 and P388 proliferation.

The above results show that antitumor T-cells were induced in L1210-bearing mice by the present therapeutic regimen. We next examined whether these antitumor T-cells were responsible for an *in situ* enhancement of the therapeutic effect in donor mice *per se*.

Effect of Anti-Mouse Thymocyte Globulin on the Therapeutic Enhancement. L1210-bearing mice treated with both 6-MP and L1210 vaccine were inoculated i.p. with rabbit anti-mouse thymocyte globulin. As shown in Table 5, the latter treatment decreased their survival time. In addition, they survived for a

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shorter period than did L1210-bearing mice treated with 6-MP alone (mean survival days, 15 *versus* 19). In contrast, the injection of normal rabbit globulin did not affect the therapeutic effect, confirming that antitumor T-cells were responsible for the enhanced therapeutic effect in donor mice.

DISCUSSION

Our previous investigation has demonstrated that treatment of L1210-bearing mice with L1210 vaccine and 6-MP resulted in an augmented therapeutic effect (8). In the present study, we examined the immunological consequence of this effect. The i.p. neutralization test showed that antitumor effector cells in the spleen and peritoneal cavity of L1210-bearing mice treated with L1210 vaccine and 6-MP, but not with either agent alone, participated in the induced therapeutic effect. We stress that, in terms of donor antitumor activity, our i.p. transfer test provides more reliable information than do the i.m. or s.c. transfer tests in which tumor cells contact immunocytes and in which the tumor surveillance process is not included (11).

The antitumor effector cells in the spleen and peritoneal cavity were identified asT-cells because their antiproliferation and neutralizing activity against L1210 cells was abolished upon their treatment with anti-Thy 1.2 antibody and complement (Tables 2 and 3). Their selectivity for L1210 cells (Table 4) and the association of antitumor activity with the nonadherent cell fraction support our conclusion. However, the present results do not eliminate the involvement of other antitumor immunocytes such as natural killer cells in the observed antitumor activity.

While the *in vivo* neutralization and the *in vitro* antiproliferation test results identified the antitumor cells to be T-cells (Tables 1 to 3), they did not prove unequivocally that T-cells eliminated L1210 cells in donors *in situ*. However, our finding that the inoculation of rabbit anti-mouse thymocyte globulin into L1210-bearing mice nullified the therapeutic effect induced by L1210 vaccine and 6-MP strongly suggests that T-cells participated importantly in the manifestation of this therapeutic effect.

It is not clear what role is played by the site of origin of the antitumor T-cells. The antitumor activity of peritoneal cells was greater than that of spleen cells on a per cell basis (Table 1). This observation was confirmed by the results of the *in vitro* antiproliferation test; at a 100:1 to 500:1 ratio of peritoneal effector cells to L1210 cells, L1210 proliferation was suppressed to the same extent as when the ratio of splenic effector cells to L1210 cells was 1000:1. Although the enriched peritoneal cells not adhering to the plastic flask were used in the *in vitro* study, the nonadherence may not affect the observed superiority of peritoneal cells over spleen cells in antitumor activity since the nonadherent fraction of peritoneal cells occupies more than 50% of the whole population. This observation may be relevant to experimental situations in which live L1210 cells are inoculated i.p. so that the antitumor effector cells proliferate in the peritoneal cavity and/or come into the peritoneal cavity from other parts of the body, *i.e.*, the spleen, and then react with L1210 cells (4).

It is not clear at present how 6-MP was associated with the production of antitumor T-cells. The elimination of L1210 cells by 6-MP could have relieved the host from immunological suppression because tumor-derived suppressive factors inhibit host antitumor immunity (7, 21). However, this would be only partially, if at all, responsible for the augmentation of host antitumor immunity since other chemotherapeutic agents, including cyclophosphamide, did not induce an augmented therapeutic effect, although they did eliminate L1210 cells to an extent similar to or greater than that of 6-MP (8).

On the other hand, we found that suppressor macrophages induced in the peritoneal cavity of L1210-bearing mice and capable of suppressing the anti-L1210 immunity in L1210 vaccine-primed mice were inhibited by 6-MP but not by the other chemotherapeutic agents including cyclophosphamide (10). These results suggest that the augmented antitumor immunity was induced in L1210-bearing mice by inhibiting the suppressor macrophages in a 6-MP-dependent fashion resulting in the augmentation of L1210 vaccine-dependent antitumor immunity.

Table 4

Tumor selectivity of peritoneal antitumor cells from L1210-bearing mice treated with 6-MP and L1210 vaccine

Peritoneal cells (Day 14 or 15) were prepared from L1210-bearing mice treated with 6-MP and L1210 vaccine as indicated in Table 2. Cells (10^6/ml) not adhering to plastic flasks were examined for antiproliferation activity. Peritoneal cells of mice immune to L5178Y were prepared according to the procedure described in "Materials and Methods" and were used without further fractionation.

	% of control tumor cell concentration[a] in the presence of peritoneal cells sensitized to		
	L1210		
Target cells	Experiment 1	Experiment 2	L5178Y
L1210	19.2 ± 2.0[b,c] (18.0 ± 1.9)[d]	23.7 ± 19.0[c]	88.5 ± 5.6
L5178Y		126 ± 8.5	27.5 ± 3.2[c]
P388	155 ± 16.9		85.5 ± 12.0

[a] Normal peritoneal cells were not present (L1210, except value designated by Footnote *d*) or present (L5178Y) in control mixtures.
[b] Mean ± SD of triplicate or more than triplicate determinations.
[c] Statistically significant at $P < 0.05$ by the *t* test as compared with other groups.
[d] Values *versus* control in which normal peritoneal cells not adhering to the plastic flask were added.

Table 5

Inoculation of rabbit anti-mouse thymocyte globulin into L1210-bearing mice nullifying the therapeutic effect of 6-MP and L1210 vaccine treatment

Mice inoculated i.p. with 2.5×10^2 live L1210 cells (Day 0) were given i.p. injections of 6-MP (100 mg/kg) (Day 5) and 10^7 L1210 cell vaccine (Days 1 and 8). They were further inoculated i.p. with 0.25 ml rabbit anti-mouse thymocyte globulin or normal rabbit globulin (Days 6, 7, 9, 11, 13, and 15).

	Survival of mice	
Treatment	No. of cured mice/total	Survival days of L1210-bearing mice
None	1/8	26 ± 6[a]
Normal rabbit globulin	3/8	21 ± 4
Rabbit anti-mouse thymocyte globulin	0/8	15 ± 1[b,c]

[a] Mean ± SD.
[b] Statistically significant at $P < 0.05$ by the Mann-Whitney test as compared with all other groups.
[c] Prolonged as compared with the untreated mice (11 ± 0), but shorter than the survival of mice treated with 6-MP alone (19 ± 2) ($P < 0.05$ by the Mann-Whitney test).

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REFERENCES

1. Bach, J-F. The Mode of Action of Immunosuppressive Agents, pp. 93–172. Amsterdam: North Holland Publishing Co., 1975.

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2. Chanmougan, D., and Schwartz, R. S. Enhancement of antibody synthesis by 6-mercaptopurine. J. Exp. Med., *124:* 363–378, 1966.
3. Ehrke, M. J., and Mihich, E. Adriamycin and other anthracyclines. *In:* J. Fahey and M. Mitchell (eds.), Clinics in Immunology and Allergy, Vol. 4, pp. 259–277. Philadelphia: W. B. Saunders Co., 1984.
4. Glaser, M., and Herberman, R. Secondary cell-mediated cytotoxic response to challenge of rats with syngeneic gross virus-induced lymphoma. J. Natl. Cancer Inst., *56:* 1211–1215, 1976.
5. Greenberg, P. D., Cheever, M. A., and Fefer, A. Eradication of disseminated murine leukemia by chemoimmunotherapy with cyclophosphamide and adoptively transferred immune syngeneic Lyt-1^+2^- lymphocytes. J. Exp. Med., *154:* 952–963, 1981.
6. Hengst, J. C. D., Mokyr, M. B., and Dray, S. Cooperation between cyclophosphamide tumoricidal activity and host antitumor immunity in the cure of mice bearing large MOPC-315 tumors. Cancer Res., *41:* 2163–2167, 1981.
7. Kamo, I., and Friedman, H. Immunosuppressive factors in cancer. Adv. Cancer Res., *25:* 271–321, 1977.
8. Kataoka, T., Akahori, Y., and Sakurai, Y. 6-Mercaptopurine-induced potentiation of active immunotherapy in L1210-bearing mice treated with concanavalin A-bound leukemia cell vaccine. Cancer Res., *44:* 519–524, 1984.
9. Kataoka, T., Kobayashi, H., and Sakurai, Y. Potentiation of concanavalin A-bound L1210 vaccine *in vivo* by chemotherapeutic agents. Cancer Res., *38:* 1202–1207, 1978.
10. Kataoka, T., and Oh-hashi, F. Suppressor macrophages in tumor-bearing mice and their selective inhibition by 6-mercaptopurine. Cancer Res., *45:* 2139–2144, 1985.
11. Kataoka, T., Oh-hashi, F., and Sakurai, Y. Identification of effector T cells responsible for enhanced antitumor immunity induced by tumor vaccine and pyran copolymer. Cancer Immunol. Immunother., *12:* 247–253, 1982.
12. Kataoka, T., Oh-hashi, F., and Sakurai, Y. Immunogenicity and amplifier cell production by tumor vaccines enhanced by concanavalin A. Gann, *73:* 193–205, 1982.
13. Kataoka, T., Oh-hashi, F., Sakurai, Y., and Ogihara, K. Effect of antineoplastic agents on the induction of suppressor macrophages by concanavalin A-bound tumor vaccine. Cancer Res., *41:* 5151–5157, 1981.
14. Maguire, H. C., Jr. Increased tumor immunity with cyclophosphamide. Proc. Am. Assoc. Cancer Res., *18:* 185, 1977.
15. Mantovani, A., Polentarutti, N., Luini, W., Peri, G., and Spreafico, F. Role of host defense mechanisms in the antitumor activity of Adriamycin and daunomycin in mice. J. Natl. Cancer Inst., *63:* 61–66, 1979.
16. North, R. J. Cyclophosphamide-facilitated adoptive immunotherapy of an established tumor depends on elimination of tumor-induced suppressor T cells. J. Exp. Med., *155:* 1063–1074, 1982.
17. Santoni, A., Riccardi, C., Sorci, F., and Herberman, R. B. Effects of Adriamycin on the activity of mouse natural killer cells. J. Immunol., *124:* 2329–2355, 1980.
18. Schwartz, R. S., and Beldotti, L. The treatment of chronic murine homologous disease: a comparative study of four immunosuppressive agents. Transplantation (Baltimore), *3:* 79–97, 1965.
19. Sendo, F., Miyake, T., Fuyama, S., and Arai, S. Spontaneous regression of syngeneic transplanted tumor in rats pretreated with the antileukemia drug busulfan. J. Natl. Cancer Inst., *60:* 385–389, 1978.
20. Stoychkov, J. N., Schultz, R. M., Chirigos, M. A., Pavlidis, N. A., and Goldin, A. Effects of Adriamycin and cyclophosphamide treatment on induction of macrophage cytotoxic function in mice. Cancer Res., *39:* 3014–3017, 1979.
21. Theofilopoulos, A. N., and Dixon, F. J. The biology and detection of immune complexes. Adv. Immunol., *28:* 89–220, 1979.

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