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Suppression and Restoration of Cytotoxic T-Cell Activity during Chemotherapy of a Mouse T-Cell Lymphoma and a Macrophage Tumor[1]

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ABSTRACT

Effects of therapy with antineoplastic chemicals on the growth of tumors and the induction of cytotoxic T-cells in the spleens of treated hosts were studied in mice bearing syngeneic tumors, J774 macrophage tumor or EL-4 T-cell lymphoma. Progressively growing tumors suppressed the capacity for *in vitro* inducibility of spleen T-cells cytotoxic to allogeneic target cells. Doses of nitrogen mustard, mitomycin C, and polyinosinic-polycytidylic acid, which decreased the growth of J774, counteracted the tumor-induced suppression of *in vitro* cytotoxic T-cell induction in spleen cells from BALB/c mice. Higher doses of nitrogen mustard and polyinosinic-polycytidylic acid inhibited the ability of spleen T-cells to become cytotoxic even in tumor-free mice. 1-β-D-Arabinofuranosylcytosine and nitrogen mustard decreased the growth of EL-4 ascites tumor and simultaneously restored the cytotoxic T-cell function of spleen cells in C57BL/6J mice. Polyinosinic-polycytidylic acid inhibited the growth of EL-4 only weakly and did not relieve the tumor-induced suppression of cytotoxic T-cell response. The highest levels of nitrogen mustard and polyinosinic-polycytidylic acid decreased cytotoxic T-cell activity in normal C57BL/6J mice.

These results demonstrate the inhibitory effects of several chemotherapeutic drugs on tumor growth and simultaneously correlate with restoration of cell-mediated immunological reactivity.

INTRODUCTION

Currently used chemotherapeutic agents primarily affect the proliferative capacity of tumor cells. However, it is well established that the immunological host defense mechanisms against tumors are also affected, depending on the nature and dose of the agent and its schedule of administration (12). These effects of chemotherapy on immune defense mechanisms of the host often lead to secondary complications because of reduced host immunity. Optimal chemotherapeutic agents or regimen of treatment should have minimal effects on the host defense systems (15, 16).

In this study results are presented showing the effect of antitumor chemotherapeutic agents on: (*a*) the growth of a T-cell lymphoma, EL-4 (9), or a macrophage tumor, J774 (20), of the mouse; and (*b*) the induction of cytotoxic T-cells in the spleens of tumor-bearing and tumor-free mice. These 2 tumors were selected because they are derived from cell compartments that would, under normal circumstances, function in host defense. Thus, we were measuring the function of an immune parameter in mice undergoing treatment for tumors of the immune system.

MATERIALS AND METHODS

Tumors. EL-4 lymphoma was grown in male C57BL/6J mice; P815 mastocytoma (7) was grown in male DBA/2J mice from The Jackson Laboratory, Bar Harbor, Maine; and the J774 macrophage tumor was grown in female BALB/cCrl mice from Charles River Breeding Laboratories, Inc., Wilmington, Mass. Tumors were maintained and tested in ascites form. Mice weighed 18 to 22 g at the time of tumor implantation and were given water and food *ad libitum*. No evidence of virus particles has been found in cells of EL-4 or J774 tumors by transmission electron microscopy (A. Liepins, personal communication). Serodiagnostic tests for 11 viruses performed by Microbiological Associates, Bethesda, Md., were negative.

The number of tumor cells present in the spleens of tumor-bearing mice was assessed by cultivating 1×10^5 spleen cells in Falcon 1001 tissue culture plates in 1 ml of 3% agar in McCoy's tissue culture medium containing 20% fetal calf serum (18). Cultures were incubated in a humidified incubator at 37° and removed on Day 5 (EL-4) or Day 12 (J774) for determination of proliferating aggregates (>3 cells). Cultures of normal spleen cells were used as controls. The plating efficiency for ascites tumor cells was 27% for J774 and 13% for EL-4 cells.

Chemicals. ara-C,[3] Mit-C, and HN2 were obtained from the Cancer Chemotherapy National Service Center, Bethesda, Md. poly(rI)·poly(rC) was prepared by Dr. Leonard D. Hamilton of the Brookhaven National Laboratory, Upton, N. Y. (11). Chemicals were dissolved in pyrogen-free 0.85% NaCl solution; solutions of HN2 were prepared fresh every day 5 to 10 min before injection.

Therapy. Drugs were administered i.p. once a day for 6 days starting the day following tumor implantation (Day 0). EL-4 cells, 1×10^6 i.p., were implanted into groups of 7 mice each, of which 5 mice were used for the determination of the TPCV of tumor cells on Day 8 and 2 mice served as

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[3] The abbreviations used are: ara-C, 1-β-D-arabinofuranosylcytosine; Mit-C, mitomycin C; HN2, nitrogen mustard; poly(rI)·poly(rC), polyinosinic-polycytidylic acid; TPCV, total packed cell volume (of ascites cells); MST, median survival time; BSS, Hanks' balanced salt solution; RPMI Medium 1640, Roswell Park Memorial Institute Tissue Culture Medium 1640.

the source of spleen cells for *in vitro* cytotoxic T-cell induction. This time was chosen since it is immediately after the end of therapy administration but soon enough before untreated, tumor-bearing mice begin to die or become moribund. TPCV was determined as described previously (23). Briefly, mice were killed and weighed, their peritoneal cavity was opened, and a microcapillary glass tube was filled with ascitic fluid. After centrifugation in the International Hematocrit centrifuge for 7 min at 10,000 rpm, the fraction of sample volume occupied by packed tumor cells (*Fc*) was determined with a Cytocrit microcapillary reader. Ascitic fluid was drained from the peritoneal cavity, which was then dried with absorbent paper, and the mouse carcass was reweighed. Ascites volume (*Va*) was calculated as the difference between the body weight with and without the ascitic fluid (the density of ascitic fluid was assumed to be 1). TPCV was then calculated by the formula TPCV (ml) = $Fc \times Va$. The average TPCV from each group of 5 mice was calculated on the basis of individual TPCV's. In addition to the 7 tumor-bearing mice, 2 tumor-free C57BL/6J mice were treated in the same manner as the corresponding tumor-bearing mice; spleens of such mice served as the source of normal cytotoxic T-cells.

Test groups of J774 consisted of 8 tumor-bearing and 2 tumor-free mice. Mice were inoculated on Day 0 with 1×10^6 J774 cells i.p., and therapy was administered in the same way as for the EL-4 mice. The volume of J774 in the peritoneal cavity of the mice was sufficient to maintain the tumor in ascites form but was too small to allow an accurate measurement of the TPCV. Therefore, the antitumor effect was evaluated by the MST of 6 treated tumor-bearing mice from each group. The remaining 2 tumor-bearing and 2 tumor-free mice were used as the source of normal spleen cytotoxic T-cells.

***In Vitro* Cytotoxic T-Cell Induction and Cytotoxicity Assay.** The *in vitro* induction of T-cells cytotoxic against allogeneic tumor cells was performed as follows (5). Spleens from mice were aseptically removed and teased in 10 ml of BSS. Cell suspensions were aspirated into plastic tubes and incubated for 5 to 10 min at 0° to allow the debris to settle. The supernatants containing cells were aspirated and centrifuged for 5 min at 450 × *g* at 0°. Cells were then washed 3 times with BSS and suspended in Click's medium (4) containing 5% specially screened fetal calf serum (Microbiological Associates). EL-4 cells served as stimulating alloantigens for the spleen T-cells from J774-bearing test mice, as did P815 cells for the spleen cells from EL-4-bearing mice. These stimulator cells were collected from the ascites of tumor-bearing mice and exposed for 30 min at 37° to 0.5 mg Mit-C per 10^6 tumor cells in a volume of 10 ml BSS with 10% calf serum.

Spleen cells were then mixed with their allogeneic stimulator cells at a final concentration of 1×10^7 or 5×10^5 cells/ml, respectively. Aliquots of 1 ml were then dispensed into 35-mm tissue culture dishes (Falcon Plastics, Oxnard, Calif.) and rocked for 5 days at 37° in a CO_2 air incubator. At the end of the incubation, spleen cells were harvested, washed in BSS, and suspended in RPMI Medium 1640 containing 10% calf serum.

Lymphocyte-mediated cytotoxicity was assayed as previously described (7). Briefly, target cells were collected from mice bearing 7-day-old ascites tumors, washed with RPMI Medium 1640 containing 10% calf serum, and incubated in the same medium containing 200 μCi $Na_2{}^{51}CrO_4$ (specific activity, 300 to 400 μCi/mg) (New England Nuclear, Boston, Mass.) at a cell density of 5 to 8×10^6 cells/ml. The incubation lasted 45 min at 37° in a humidified CO_2-air incubator. Excess chromium label and cell debris were removed by layering cell suspensions over 10 ml of RPMI Medium 1640 containing 20% calf serum and centrifuging for 3 min at 200 × *g*.

Following a modified procedure of Brunner *et al.* (2), mixtures of 40×10^5 spleen cells and 1×10^5 labeled target cells in a 0.5-ml volume were incubated in 35-mm Falcon plastic Petri dishes on a rocking platform in a humidified incubator. At 4 hr the entire content of one Petri dish was transferred into a centrifuge tube and centrifuged for 7 min at 450 × *g*. The ^{51}Cr activity of the supernatant was determined in a γ scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.). Specific ^{51}Cr release was calculated by the formula:

$$\%\ \text{specific cytotoxicity} = \frac{\text{Experimental } {}^{51}\text{Cr release} - \text{spontaneous } {}^{51}\text{Cr release}}{\text{Total } {}^{51}\text{Cr release} - \text{spontaneous } {}^{51}\text{Cr release}} \times 100$$

RESULTS

Chemicals used in this study were selected from a larger group of chemotherapeutic and immunomodulating agents that have shown antitumor activity against EL-4 or J774 in our ongoing survey of the effectiveness of agents against tumors of the lymphoid origin in the mouse (to be published).

Treatment of J774 with Mit-C, HN2, or poly(rI)·poly(rC) (Table 1) resulted in a dose-dependent prolongation of the MST of treated hosts; with the highest dose of Mit-C, the MST decreased. In mice treated only with 0.85% NaCl solution, the presence of J774 tumor reduced the ability of spleen T-cells to be induced to cytotoxicity toward allogeneic tumor targets *in vitro*. The capacity for *in vitro* induction of cytotoxic spleen T-cells was restored in mice treated with increasing doses of the therapeutic chemicals. The highest doses [Mit-C, 3 mg/kg/day; HN2, 1 mg/kg/day; and poly(rI)·poly(rC), 10 mg/kg/day] significantly reduced the capacity for *in vitro* induction of cytotoxic T-cells as evidenced by low levels or absence of specific ^{51}Cr release from target cells incubated with cultured spleen cells from these mice. These highest doses of HN2 and poly(rI)·poly(rC) also greatly reduced the capacity for *in vitro* cytotoxic T-cell induction in the spleens of tumor-free mice. No such suppression was observed in tumor-free mice treated with the highest dose of Mit-C. In preliminary experiments therapy with ara-C did not significantly prolong the survival times of mice bearing J774 tumor (Table 1), and immune parameters were not studied.

In mice bearing EL-4 lymphoma, treatment with ara-C (Table 2) produced a response similar to that observed in mice bearing J774 that were treated with Mit-C. There was a dose-dependent inhibition of tumor growth measured as

Table 1

Effect of chemotherapy on growth of J774 macrophage tumor and on the in vitro induction of specific cytotoxic T-cells in mouse spleen cell suspensions

J774 cells, 1×10^6, were injected i.p. on Day 0 into each mouse in groups of 8 mice/dose of chemical. The therapy was 6 i.p. doses, one/day, beginning on Day 1. Six mice were observed for 56 days to determine the MST. Two tumor-bearing mice and 2 similarly treated tumor-free mice were sacrificed on Day 8 for the *in vitro* induction of cytotoxic spleen T-cells as described in "Materials and Methods."

		Tumor-bearing mice		Cytotoxic spleen T-cells (% specific ^{51}Cr release)	
Chemical	Dose (mg/kg/day)	MST (days)	Survivors at Day 56	Tumor-bearing mice	Tumor-free mice
Mit-C	0	16.2 ± 1.1[a]	0	8.7 ± 1.4[b]	31.1 ± 1.1[b]
	0.1	21.3 ± 1.8[c]	0	21.9 ± 5.2	25.8 ± 0.5[d]
	0.3	19.3 ± 0.8[c]	0	27.8 ± 0.9[d]	29.2 ± 4.1
	1	32.5 ± 5.2[c]	1	29.7 ± 1.0[d]	25.9 ± 2.6
	3	14.3 ± 1.6	0	14.7 ± 0.2	35.5 ± 5.9
HN2	0	17.5 ± 1.1	0	0	40.0 ± 2.6
	0.01	16.7 ± 1.3	0	0	47.7 ± 2.1
	0.03	19.4 ± 1.3	0	24.3 ± 0.7[d]	51.7 ± 1.3
	0.1	21.5 ± 1.3[c]	0	67.4 ± 2.1[d]	23.7 ± 1.0[e]
	0.3	32.3 ± 4.8[c]	1	61.4 ± 4.6[d]	61.8 ± 15.0
	1	40.2 ± 6.6[c]	2	7.0 ± 0.5[d]	0[d]
poly(rI)·poly(rC)	0	16.7 ± 1.3	0	0	75.7 ± 1.9
	0.1	23.5 ± 2.6[c]	0	1.3 ± 0.0	69.9 ± 0.7
	1	33.2 ± 5.2[c]	0	60.1 ± 2.3[d]	46.9 ± 1.3[d]
	10	49.2 ± 2.9[d]	1	0.7 ± 0.3	3.8 ± 0.3[d]
ara-C	0	20.7 ± 1.8	0	Not done	
	0.3	22.4 ± 1.4	0		
	3	26.6 ± 1.4[c]	0		
	30	29.3 ± 2.1[c]	0		

[a] Mean ± S.D.
[b] Results are the mean of 3 cultures ± S.D.
[c] Significantly different from 0.85% NaCl solution control of each group, $p < 0.05$.
[d] $p < 0.01$.
[e] $p < 0.05$; in other experiments no inhibition of induction of cytotoxic T-cells was seen in tumor-free mice treated with HN2 except at the highest dose of 1 mg/kg/day.

a decrease of TPCV on Day 8. The highest doses restored the capacity for cytotoxic T-cell induction *in vitro* in spleen cells from treated tumor-bearing mice. HN2 caused an almost complete inhibition of tumor growth in doses of 0.1 mg/kg/day and higher, the 2 higher doses were toxic and caused a body weight loss, and one death occurred among 5 mice treated with 1 mg/kg/day. The intermediate doses of HN2 released suppression of *in vitro* cytotoxic T-cell induction in spleen cells of tumor-bearing mice; the higher doses of HN2 decreased this parameter in both the tumor-bearing and tumor-free mice.

In contradistinction to J774, therapy of EL-4-bearing mice with the immunomodulator poly(rI)·poly(rC) produced only a moderate antitumor effect in doses of 1 and 10 mg/kg/day. No abrogation of the tumor-induced suppression of *in vitro* cytotoxic T-cell induction was observed at any dose; in tumor-free mice intermediate and high doses of the drug decreased the induction of cytotoxic T-cells.

The effect of chemotherapy on the number of tumor cells in spleens of tumor-bearing mice was determined by cloning tumor cells in semisolid agar cultures and counting the number of proliferating foci after several days of growth. Tumor cells were present in spleens of untreated mice (Table 3); EL-4 cells were found to be 2% and J774 cells were 0.25% of the total splenic cell content. Mit-C and HN2 lowered the number of tumor cells to less than 0.001% of all cells present in the spleen. The effects of ara-C and poly(rI)·poly(rC) on tumor load in spleens have not been quantitated as they were in the case of Mit-C and HN2; both former chemicals markedly reduced the number of J774 tumor cells in the spleens of tumor-bearing mice (data not shown).

Reduction of the tumor colony-forming capacity by chemotherapy shows correlation with the restoration of cellular immune response. The total number of spleen cells recovered from mice was not grossly affected by tumor injection or chemotherapy, except at high drug doses.

DISCUSSION

Clinically useful antitumor chemicals are known to suppress significantly the immune responses of the hosts and to affect both the antibody formation and cellular immune responses (3, 8, 12–16). It seems paradoxical to treat cancer

Table 2

Effect of chemotherapy on growth of EL-4 lymphoma and on the in vitro induction of specific cytotoxic T-cells in mouse spleen cell suspensions

Tumor implantation (in groups of 7 mice) and therapy were as in Table 1. On Day 8, 5 mice were sacrificed for the TPCV measurement; 2 tumor-bearing mice and 2 similarly treated tumor-free mice were sacrificed for *in vitro* induction of cytotoxic spleen T-cells. In other experiments untreated mice bearing EL-4 tumor died with MST's of 13 to 19 days; the MST increased more than 2-fold after therapy with ara-C and HN2, but not Mit-C.

		Tumor-bearing mice on Day 8		Cytotoxic spleen T-cells (% specific ^{51}Cr release)	
Chemical	Dose (mg/kg/day)	TPCV (ml)	AWC[a] (g)	Tumor-bearing mice	Tumor-free mice
ara-C	0	0.52 ± 0.03[b]	−0.8	1.6 ± 0.3[c]	58.6 ± 0.3[c]
	0.1	0.49 ± 0.03	−0.8	3.6 ± 0.7[d]	40.9 ± 1.1[d]
	0.3	0.13 ± 0.02[d]	+0.8	5.3 ± 0.1[d]	34.2 ± 0.5[d]
	1	0[d]	+0.2	12.4 ± 0.5[d]	44.7 ± 1.8[e]
	3	0[d]	+0.3	38.8 ± 0.7[d]	25.6 ± 0.1[d]
HN2	0	0.59 ± 0.08	+0.4	0	38.5 ± 7.8
	0.01	0.28 ± 0.05[e]	+0.7	0	26.9 ± 1.6
	0.03	0.06 ± 0.03[d]	+0.3	23.0 ± 1.1[d]	25.2 ± 1.3
	0.1	0.01 ± 0[d]	−0.5	19.3 ± 3.7[e]	39.2 ± 2.8
	0.3	0.01 ± 0[d]	−2.0	16.6 ± 1.1[d]	10.5 ± 2.6
	1	0.01 ± 0.01[d]	−5.4	14.2 ± 1.1[d]	0.5 ± 0.75[e]
poly(rI)·poly(rC)	0	0.59 ± 0.08	+0.4	0	38.5 ± 7.8
	0.1	0.63 ± 0.04	−0.7	0	22.3 ± 1.0
	1	0.26 ± 0.09[e]	−0.8	0	15.4 ± 1.7
	10	0.22 ± 0.08[e]	−2.4	0	4.6 ± 1.4[e]

[a] AWC, average weight change between Days 0 and 8.
[b] Mean ± S.D.
[c] Results are the mean of 3 cultures ± S.D.
[d] $p < 0.01$.
[e] $p < 0.05$.

Table 3

Effect of chemotherapy of J774 and EL4 on MST, cytotoxic T-cell induction, and splenic tumor load

Tumor implantation and therapy were as in Table 1. *In vitro* induction of cytotoxic spleen T-cells and measurement of splenic tumor load were as described in "Materials and Methods."

			Tumor-bearing mice				
				Cytotoxic spleen T-cells[a] (% specific ^{51}Cr release)		Spleen content[a]	
Tumor	Chemical	Dose (mg/kg/day)	MST (days)	Tumor-bearing mice	Tumor-free mice	Total cells ×10^{-6}	Tumor cells ×10^{-3}
J774	Mit-C	0.9% NaCl solution	16.2 ± 1.6[b]	0	23.1 ± 5.5	80	209 ± 8[c]
		0.1	17.3 ± 1.8	0	25.7 ± 1.0	100	115 ± 13[d]
		0.3	18.3 ± 2.0	9.2 ± 1.9[d]	23.5 ± 3.8	73	23 ± 6[d]
		1	18.1 ± 2.9	26.5 ± 1.1[d]	23.0 ± 2.4	68	1 ± 1[d]
		3	26.8 ± 2.4[d]	46.7 ± 1.0[d]	38.9 ± 3.6[d]	24	0[d]
EL-4	HN2	0.9% NaCl solution	11.6 ± 0.8	0	42.1 ± 6.1	50	922 ± 46
		0.01	14.6 ± 2.0[e]	0	66.5 ± 2.7[d]	63	50 ± 7[d]
		0.03	17.5 ± 2.5[d]	5.7 ± 1.3[d]	30.1 ± 1.7[d]	63	6 ± 2[d]
		0.1	18.3 ± 1.5[d]	4.6 ± 2.9[d]	31.5 ± 7.8	88	0[d]
		0.3	20.7 ± 2.4[d]	7.9 ± 2.9[d]	13.0 ± 1.2[d]	68	0[d]

[a] Results are the mean of 3 cultures ± S.D.
[b] Mean ± S.D.
[c] Total number of tumor cells was obtained by multiplying the number of cloned tumor cells by plating efficiency.
[d] $p < 0.01$.
[e] $p < 0.05$.

with drugs that profoundly inhibit the very system considered essential for protection against neoplastic cells. However, not all chemicals are immunosuppressive, and in the case of the immunosuppressive drugs their powerful antitumor activity is believed to counterbalance their suppression of general and/or specific antitumor immunity (24).

In this study effects of several antitumor chemicals were investigated in mice bearing J774 or EL-4 tumors. Tumor growth and the capacity for cytotoxic T-cell induction against allogeneic tumor cells in cultures of spleen cells obtained from treated tumor-bearing mice were examined. Cytotoxic T-cell induction was selected for this study because of the relative paucity of information in the literature on this subject and because of the suspected role of cytotoxic T-cells in immune surveillance. Effects of chemotherapy of animal and human tumors on the antibody response of tumor hosts to different antigens and on such parameters of the cell-mediated response as delayed cutaneous hypersensitivity, graft rejection, and responsiveness of lymphocytes to lectins have been subjects of numerous studies and have been repeatedly reviewed (12–16). On the other hand only a few studies were performed that monitored the effects of chemotherapy on different components of the cytotoxic T-cell response of the host.

Our studies reveal 3 salient points relevant to effective chemotherapy. First, rapidly growing tumors suppress cell-mediated immune responses to allogeneic cells, as reviewed by Kamo and Friedman (17). Second, some chemotherapeutic agents can inhibit tumor growth and concomitantly restore the suppressed immunity. Third, chemotherapeutic agents, when administered in too high doses, reduce tumor load but also cause immune dysfunction.

In our experiments 2 types of responses were observed. ara-C and HN2 in EL-4-bearing mice and Mit-C and HN2 in J774-bearing hosts decreased in a dose-dependent manner the number of tumor cells in the spleens and the total packed tumor cell volume of EL-4 and increased the MST of J774-bearing mice. The tumor-induced suppression of cytotoxic T-cells in the spleen was abrogated in parallel with the drug-dependent decrease of the existing tumor load. The highest doses of Mit-C and HN2 produced a decrease of cytotoxic T-cell activity as evidenced by the reduced specific ^{51}Cr release from target cells. However, only the highest dose of HN2 abolished the induction of cytotoxic T-cells in the spleens of tumor-free mice. ara-C, even in the highest tested dose, did not decrease the induction of cytotoxicity of spleen T-cells in either tumor-bearing or tumor-free mice.

The second type of response was observed with poly(rI)·poly(rC). In the higher doses the chemical markedly decreased the capacity for *in vitro* induction of cytotoxic T-cells in spleens of tumor-free mice, and at any tested dose it did not release the suppressed inducibility in EL-4 tumor-bearing mice. In mice bearing J774 the intermediate, but not the highest, dose of chemical released the suppression of cytotoxic T-cell induction caused by tumor growth.

Heppner and Calabresi (14) treated C3H/HeJ mice bearing early-generation passages of a spontaneous mammary tumor with ara-C using doses of 10 to 40 mg/kg/day. They observed, using microcytotoxicity tests, that low drug doses abrogated the inhibition by serum-blocking factors of lymph node cell cytotoxicity. The highest dose of ara-C used in the present study was 3 mg/kg/day, and abrogation of tumor-induced suppression of inducibility of cytotoxic T-cells in spleen cultures from treated mice was obtained with a dose as low as 1 mg/kg/day, which inhibited the growth of EL-4 during the treatment period. Mice ultimately died of progressing tumors. Griswold *et al.* (10) reported that administration of ara-C to C57BL/6 mice for the first 5 days after immunization inhibited the development of humoral, but not cellular, immunity to allogeneic tumor cells.

Röllinghoff *et al.* (21) studied the *in vitro* induction of cytotoxic T-cells in cultures of spleen of CBA ($H\text{-}2^k$) mice immunized by cocultivation with Mit-C-treated BALB/c ($H\text{-}2^d$) cells. Cytotoxicity directed against P815 ($H\text{-}2^d$) cells was inhibited 50% when ara-C, 0.25 μg/ml, was present in the culture during induction; the B-cell-dependent response against dinitrophenylated flagella was inhibited 50% by 0.35 μg of this chemical per ml. Otterness and Chang (19) obtained lysis of EL-4 cells by cytotoxic spleen cells from BALB/c mice immunized with EL-4 cells; lysis was stimulated when a single dose of cyclophosphamide was administered to mice on Day 0, but the humoral response was suppressed. High doses of cyclophosphamide or treatment with azathioprine, 6-mercaptopurine, or methotrexate inhibited both the cellular and humoral responses at all levels tested. Dennert *et al.* (6) administered cyclophosphamide or 1,2-bis(3-dioxopiperazine-1-yl)ethane (Imperial Cancer Research Fund 154) i.p. to mice before or simultaneously with allogeneic P815 tumor cells. The percentage of Thy-1-positive cells and the cell-mediated cytotoxicity in the spleens of treated mice were enhanced; if therapy started shortly after injection of allogeneic cells, the generation of killer T-cells was inhibited. The reduction of cytotoxic T-cell generation encountered when cyclophosphamide is administered after antigen stimulation is probably a result of cyclophosphamide-resistant suppressor accumulation (1). Contrariwise, cyclophosphamide treatment prior to antigenic stimulation results in suppressor-cell removal (22). In devising optimal protocols for chemotherapy, specific immune mechanisms believed to contribute to host defense against tumors should be investigated in parallel with drug effects on tumor load. Direct suppression of host responses by tumor cells or tumor-related induction of host suppressor cells must be taken into account, as well as the effect of therapy on the suppression mechanisms.

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REFERENCES

1. Bonavida, B. Antigen-Induced Cyclophosphamide-Resistant Suppressor T-Cells Inhibit the *in Vitro* Generation of Cytotoxic Cells from One-Way Mixed Leukocyte Reactions. J. Immunol., *119:* 1530–1533, 1977.
2. Brunner, K. T., Mauel, J., Rudolf, H., and Capuis, B. Studies of Allograft Immunity in Mice. I. Induction, Development and *in Vitro* Assay of Cellular Immunity. Immunology, *18:* 501–515, 1970.
3. Camiener, G. W., and Wechter, W. J. Immunosuppression—Agents, Procedures, Speculations and Prognosis. Progr. Drug Res., *16:* 67–156, 1972.
4. Click, R. E., Benck, L., and Alter, B. J. Immune Responses *in Vitro*. I.

Culture Conditions for Antibody Synthesis. Cellular Immunol., *3:* 264–276, 1972.
5. Dennert, G., and Hatlen, L. E. Induction and Properties of Cytotoxic T-Cells Specific for Hapten-Coupled Tumor Cells. J. Immunol., *114:* 1705–1712, 1975.
6. Dennert, G., Hatlen, L. E., and Tucker, D. F. Effects of Two Bisdioxopiperazines on Mouse B- and T-Cell Function. J. Natl. Cancer Inst., *54:* 621–629, 1975.
7. Faanes, R. B., Choi, Y. S., and Good, R. A. Escape from Isoantiserum Inhibition of Lymphocyte Mediated Cytotoxicity. J. Exptl. Med., *137:* 171–182, 1973.
8. Gabrielson, A., and Good, R. Chemical Suppression of Adaptive Immunity. Advan. Immunol., *6:* 91–229, 1967.
9. Gorer, P. A. Studies in Antibody Response of Mice to Tumour Inoculation. Brit. J. Cancer, *4:* 372–379, 1950.
10. Griswold, D. E., Heppner, G. H., and Calabresi, P. Selective Manipulation of Immunological Responses with Cytosine Arabinoside. Federation Proc., *29:* 431, 1970.
11. Hamilton, L. D. Immunogeneic Polynucleotides. *In:* R. F. Beers, Jr. and W. Braun (eds.), Biological Effects of Polynucleotides, pp. 107–128. Berlin: Springer-Verlag, 1971.
12. Haskell, C. M. Immunologic Aspects of Cancer Chemotherapy. Ann. Rev. Pharmacol. Toxicol., *17:* 179–195, 1977.
13. Heppner, G. H., and Calabresi, P. Suppression by Cytosine Arabinoside of Serum-Blocking Factors of Cell-Mediated Immunity to Syngeneic Transplants of Mouse Mammary Tumors. J. Natl. Cancer Inst., *48:* 1161–1167, 1972.
14. Heppner, G. H., and Calabresi, P. Selective Suppression of Humoral Immunity by Antineoplastic Drugs. Ann. Rev. Pharmacol. Toxicol., *16:* 367–379, 1976.
15. Hersh, E. M. Modification of Host Defense Mechanisms. *In:* J. F. Holland and E. Frei, III (eds.), Cancer Medicine, pp. 681–699. Philadelphia: Lea and Febiger, 1973.
16. Hersh, E. M. Immunosuppressive Agents. *In:* A. C. Sartorelli and D. G. Johns (eds.), Antineoplastic and Immunosuppressive Agents, Part 1, pp. 577–617. Berlin: Springer-Verlag, 1974.
17. Kamo, I., and Friedman, H. Immunosuppression and the Role of Suppressive Factors in Cancer. Advan. Cancer Res., *25:* 271–321, 1977.
18. Metcalf, D., and Moore, M. A. S. Haemopoietic Cells, pp. 33–35. Amsterdam: North Holland Publishing Co., 1971.
19. Otterness, I. G., and Chang, Y-H. Comparative Study of Cyclophosphamide, 6-Mercaptopurine, Azathiopurine and Methotrexate. Clin. Exptl. Immunol., *26:* 346–354, 1976.
20. Ralph, P., Prichard, J., and Cohn, M. Reticulum Cell Sarcoma: An Effector Cell in Antibody-Dependent Cell-Mediated Immunity. J. Immunol., *114:* 898–905, 1975.
21. Röllinghoff, M., Schrader, J., and Wagner, H. Effect of Azathioprine and Cytosine Arabinoside on Humoral and Cellular Immunity *in Vitro*. Clin. Exptl. Immunol., *15:* 261–269, 1973.
22. Röllinghoff, M., Starzinski-Powitz, A., Pfizenmaier, K., and Wagner, H. Cyclophosphamide-Sensitive T Lymphocytes Suppress the *in Vivo* Generation of Antigen-Specific Cytotoxic T Lymphocytes. J. Exptl. Med., *145:* 455–459, 1977.
23. Schmid, F. A., Cappuccino, J. G., Merker, P. C., Tarnowski, G. S., and Stock, C. C. Chemotherapy Studies in an Animal Tumor Spectrum. I. Biologic Characteristics of the Tumors. Cancer Res., *26* (Part 2): 173–180, 1966.
24. Schwartz, R. S. Are Immunosuppressive Anticancer Drugs Self-Defeating? Cancer Res., *28:* 1452–1454, 1968.