ABSTRACT

Specific immunological and hematopoietic functions were studied during treatment with antineoplastic agents in mice bearing syngeneic lymphoid tumors: 70Z/2, a B-cell lymphoma of C57BL x DBA/2 F1 (hereafter called BD2F1) mice; EL4, a T-cell lymphoma of C57BL/6 mice; or J774, a macrophage tumor of BALB/c mice. Both B- and T-lymphocyte function (antibody-forming cells and cell-mediated lympholysis toward alloantigens) were suppressed in spleen cells of mice bearing these tumors. Other hematopoietic functions (granulocyte, macrophage, and megakaryocyte progenitor cells) were variably influenced by growth of these lymphoid tumors. J774 enhanced, but 70Z/2 suppressed, megakaryocyte progenitor cells. J774 and 70Z/2 increased levels of granulocyte-macrophage progenitor cells. EL4, the T-cell lymphoma, did not influence either cell type. Significant variation in strain sensitivity to drug toxicity and drug effectiveness in different tumor-host systems was observed. Increased median survival time with reversal of tumor-induced immune dysfunction, without toxicity to hematopoietic progenitor cells, was realized in two tumor-host-drug combinations. Polyinosinic-polycytidylic acid was effective against 70Z/2 or J774, while actinomycin D was active against 70Z/2. Mitomycin C effectively reduced tumor load, as evidenced by loss of splenic tumor colony-forming cells for all three tumors. This agent prolonged survival and concomitantly restored immunological responsiveness in hosts immunosuppressed by growth of 70Z/2 or J774. Paralleling tumor reduction with mitomycin C therapy, the splenic hematopoietic progenitor and colony-forming B-cells were reduced in tumor-bearing and tumor-free mice, thus compromising its therapeutic effectiveness.

INTRODUCTION

Lymphoid functions have been found to be markedly suppressed in humans and laboratory animals bearing tumors (19, 33, 40, 41, 43, 47, 48). That antitumor agents themselves are immunosuppressive (4, 24) results in further compromise of host defense during therapy for malignant disease. This extensive reduction in lymphoid and hematopoietic responsiveness is a major threat to the patient because of increased susceptibility to infections which are severe and often fatal (2). We previously reported (46) that mice bearing tumors originating from the T-lymphocyte or macrophage compartment of the immune system significantly suppressed the capacity of the host for generating CTLs in vitro. These tumors differed in their susceptibility to several antineoplastic agents. Administration of Mit-C, ara-C, or nitrogen mustard significantly reduced tumor load. Concomitant with reduction of tumor load, the capacity to generate CTL was restored, and median survival time was increased.

Most defenses against infection, however, do not reside in a single lymphoid compartment. Humoral antibody responses appear to be more prone than do T-cell functions to toxic effects of anticancer drugs (6, 16, 22, 34, 45). In this study, we simultaneously evaluated B- and T-cell function during therapy. Specificity of drug-induced splenic dysfunction was determined by quantitating hematopoietic capacities in spleens during therapy. These studies were undertaken to find therapeutic regimes that would differentially reduce the lymphoid leukemia without measurable toxicity to the normal immune or hematopoietic functions.

MATERIALS AND METHODS

Tumors. EL4 lymphoma (11) was maintained and tested as ascites in male C57BL/6J mice, P815-X2 mastocytoma (9) in male DBA/2J mice, and J774 histiocytic lymphoma (37) in female BALB/c Crl mice. 70Z/2 was induced with methylnitrosoureia in a thymectomized C57BL x DBA/2 F1 (hereafter called BD2F1) mouse (1). It contains cytoplasmic immunoglobulin4 and is similar to the B-lymphoma 70Z/3 (35). C118 myeloma of the C3H mouse was obtained from G. Dennert (Salk Institute, La Jolla, Calif.) and maintained in Dulbecco's modified Eagle's medium containing 10% horse serum. C57BL/6 and DBA/2 mice were obtained from The Jackson Laboratory, Bar Harbor, Maine, BD2F1 mice were from ARS/
individual quadruplicate cultures was monitored for 51Cr activ
after a second mixing to equilibrate released 61Cr, followed by
culture for 5 hr. The extent of target cell lysis was determined
a 5-day culture period. The contents of individual wells were
seeded in Linbro No. 76-014-05) round-bottom microtiter
plates and cultured in a humidified CO2 incubator. The level of
anti-SRBC PFC were determined on the fourth day by a modi-
ted RFC-forming cell assay. Mouse spleen cells were
in vitro cloning of CFU-B, CFU-M, and CFU-C was accomplished by culturing the spleen
cells used for the immunological assays in semisolid agar.
CFU-B were counted on Day 6 from cultures of 2 x 10^6 spleen cells incubated with 5 x 10^{-5} M ß-ME, 1% SRBC, and 10 μg
of lipopolysaccharide in 1 ml of McCoy’s tissue culture medium
containing 20% FCS and 0.3% agar (21, 27). CFU-M were clonned by culturing 2 x 10^6 spleen cells in 1 ml of McCoy’s
modified Medium 5A containing 15% FCS in 0.25% Bacto agar
and nutritional supplements. For CFU-M, 2 growth activities
have been shown to be required for quantitation (50). These
entities were obtained for these studies from concentrates of
conditioned media from cultures of the murine myelomonocytic
leukemic cell line (WEHI-3) and from supernatants of cultured
macrophages. The entity of WEHI-3-conditioned medium is
obligatory for colony growth, while the macrophage superna-
tant did not directly stimulate but potentiated CFU-M numbers.
CFU-M were grown in cultures containing 100 μl of concentra-
tions of WEHI-3-conditioned medium and 200 μl of the mac-
rophage supernatant. Cultures were scored at x40 magnifi-
cation for CFU-M after 6 or 7 days of incubation at 37° in a
humidified incubator in an atmosphere of 7% CO2 in air.
Megakaryocyte colonies were readily identifiable and could be
distinguished from other colony types by the extremely large
size of the majority of the cells contained in them. Cytochemical
identification of megakaryocytes was obtained with isolated
colony cells by staining for the presence of acetylcholinester-
ase (32).

CFU-C were grown using the same conditions as for the
CFU-M except that 0.3% Bacto agar was used, and colony
formation was stimulated by concentrates of medium condi-
tioned by L-cells. Cultures were scored at day 7 (44).

Preparation of Colony-stimulating Activities. The WEHI-3-
conditioned medium was prepared from supernatants of the
cultured cells which were subcultured at 5 x 10^6 cells/ml in
25 ml in 75-sq cm plastic flasks in McCoy’s Medium 5A with
2% FCS and 5 x 10^{-5} M ß-ME. The medium was collected
after 3 or 4 days, centrifuged at 3000 x g for 5 min, and stored
frozen at -20°. Large volumes were concentrated 5-fold by
filtration with Amicon PM10 filters. Since neutrophil and
megakaryocyte stimori were found to be preferentially dimin-
ished by dialysis, this step was omitted. Polyethylene glycol
(0.001%) was added to all preparations (44). A single prepa-
ration was used in these experiments, and 100 μl of the 5-fold
concentrate was generally used.

Therapy of Tumor-induced Splenic Immune Dysfunction

The number of tumor cells present in the spleens of tumor-
bearing mice was assessed by culturing 1 x 10^6 spleen cells
in Falcon 1001 tissue culture plates in 1 ml of 0.3% agar in
McCoy’s tissue culture medium containing 20% FCS (46).
Cultures were incubated in a humidified incubator at 37°, and
the number of tumor colonies was counted on Day 5 (EL4),
Day 7 (70Z/2), or Day 12 (J774). B-lymphocytes and 70Z/2
required 5 x 10^{-5} M ß-ME for colony growth, and spleen cells
from 70Z/2-bearing mice were plated with rabbit anti-IgM
serum to eliminate growth of normal B-colony-forming cells
(21). The other tumor cells grew without exogenous stimuli.

The percentage of plating efficiency of ascites tumor cells was
26.8 ± 1.6, 13.5 ± 1.1, and 14.3 ± 0.9 (S.E.) for J774, EL4,
and 70Z/2, respectively.

LDH Virus. Plasma LDH levels were determined on heparin-
ized aliquots of plasma from mice bled at sacrifice. LDH activity
was monitored, and activities were expressed as described by
Riley (38). The tumor-free mice of the strains used were con-
sistently LDH free. Of the 3 tumors used in this study, only EL4
significantly increased plasma LDH during activity growth in
vivo, suggesting the presence of LDH virus. Attempts to free
this tumor line of LDH virus by passage in immunosuppressed
rats or in the tissue culture (30) failed to decrease LDH in the
plasma of EL4-bearing animals. 70Z/2 and J774 remained
LDH free throughout these studies. The tumors were free of
other passenger viruses, as assayed by Microbiological Asso-
ciates, Bethesda, Md.

In Vitro PFC-forming Cell Assay. Mouse spleen cells were
prepared in a manner similar to that described by Mishell and
Dutton (28) and cultured in Roswell Park Memorial Institute
Medium 1640 supplemented with 10% specifically screened,
heat-inactivated FCS and 5 x 10^{-5} M ß-ME. Generally, 1 to 2
x 10^7 spleen cells/ml were cultured in 35- x 10-mm (Falcon
No. 3001) plastic Petri dishes with an optimal number (5 x
10^6) of SRBC (Grand Island Biological Co., Grand Island,
N. Y.) as antigen. The cultures were incubated at 37° in Dutton
tissue culture chambers on a rocking platform in a humidified
incubator at 37°, and

Experimental 51Cr release - spontaneous 51Cr release
Total 51Cr release - spontaneous 51Cr release x 100

Hematopoietic Colony Assays. In vitro cloning of CFU-B,
CFU-M, and CFU-C was accomplished by culturing the spleen
cells for the immunological assays in semisolid agar.
CFU-B were counted on Day 6 from cultures of 2 x 10^6 spleen cells incubated with 5 x 10^{-5} M ß-ME, 1% SRBC, and 10 μg
of lipopolysaccharide in 1 ml of McCoy’s tissue culture medium
containing 20% FCS and 0.3% agar (21, 27). CFU-M were clonned by culturing 2 x 10^6 spleen cells in 1 ml of McCoy’s
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and nutritional supplements. For CFU-M, 2 growth activities
have been shown to be required for quantitation (50). These
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conditioned media from cultures of the murine myelomonocytic
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tant did not directly stimulate but potentiated CFU-M numbers.
CFU-M were grown in cultures containing 100 μl of concentra-
tions of WEHI-3-conditioned medium and 200 μl of the mac-
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cation for CFU-M after 6 or 7 days of incubation at 37° in a
humidified incubator in an atmosphere of 7% CO2 in air.
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size of the majority of the cells contained in them. Cytochemical
identification of megakaryocytes was obtained with isolated
colony cells by staining for the presence of acetylcholinester-
ase (32).

CFU-C were grown using the same conditions as for the
CFU-M except that 0.3% Bacto agar was used, and colony
formation was stimulated by concentrates of medium condi-
tioned by L-cells. Cultures were scored at day 7 (44).

Preparation of Colony-stimulating Activities. The WEHI-3-
conditioned medium was prepared from supernatants of the
cultured cells which were subcultured at 5 x 10^6 cells/ml in
25 ml in 75-sq cm plastic flasks in McCoy’s Medium 5A with
2% FCS and 5 x 10^{-5} M ß-ME. The medium was collected
after 3 or 4 days, centrifuged at 3000 x g for 5 min, and stored
frozen at -20°. Large volumes were concentrated 5-fold by
filtration with Amicon PM10 filters. Since neutrophil and
megakaryocyte stimuli were found to be preferentially dimin-
ished by dialysis, this step was omitted. Polyethylene glycol
(0.001%) was added to all preparations (44). A single prepa-
ration was used in these experiments, and 100 μl of the 5-fold
concentrate was generally used.

Colony-stimulating activity in L-cell-conditioned medium was
prepared from L-cells grown in the same manner as WEHI-3.
After centrifugation, the L-cell-conditioned medium was di-
alyzed overnight against 2 changes of distilled water and
concentrated 5-fold by filtration with Amicon PM10 filters. This
preparation was titrated for biological activity, and plateau
levels of L-cell-conditioned medium were used in all experi-
ments. Greater than 90% of all the colonies stimulated by L-
cell-conditioned medium were macrophages at Day 7 of cul-
ture, the remainder being mixed macrophage-neutrophil colo-
nies.
Potentiating activity for megakaryocyte colony formation was obtained from macrophage supernatants (49). Macrophages were obtained from the peritoneal cavity of C57BL/6 mice which had been given injections of 1 ml of mineral oil i.p. 4 days earlier (31). The peritoneal cells were incubated at 37° for 3 days in the absence of PCS in 25-sq mm Corning flasks 2 x 10⁶ cells/ml, after which the supernatants were removed, pooled, centrifuged, and assayed for potentiating activity.

Chemotherapy. Act-D, ara-C, and Mit-C 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. Chemicals were dissolved in pyrogen-free 0.85% NaCl solution.

Drugs were administered i.p. once a day for 6 days, starting the day following tumor implantation (Day 0). Tumor cells (1 x 10⁶) were implanted i.p. into test groups consisting of 8 mice, of which 2 mice served as the source of spleen cells for investigating immune and hematopoietic capacities. Antitumor effect was evaluated by the MST of 6 treated tumor-bearing mice from each group. The spleen donor animals were sacrificed the day following the final injection. This was immediately after the end of therapy, but soon enough before untreated tumor-bearing mice begin to die or become moribund.

RESULTS

Suppression of Splenic T- and B-Cell Functions in Mice Bearing Syngeneic Lymphoid Tumors. We previously reported (46) that spleen cells from mice bearing syngeneic lymphomas were significantly reduced in their capacity to produce CTL against alloantigens in vitro. In this study, we investigated the in vitro production of anti-SRBC IgM in parallel with the generation of CTL. Table 1 shows that all lymphoid tumors tested concomitantly suppress host capacity for in vitro antibody as well as CTL responses. In contrast, the tumor types had variable effects on hematopoiesis, as determined by assessing progenitor cell levels of granulocytes, macrophages, and megakaryocytes. Granulocytes and macrophages arise from a common precursor cell, the CFU-C. CFU-M are a separate precursor cell population giving rise to colonies comprised predominantly of megakaryocytes. The T-lymphoma (EL4) did not influence hematopoietic progenitor cell levels (Table 2). The macrophage cell line (J774) elevated CFU-C and CFU-M levels. In contrast, the B-lymphoma (70Z/2) influenced the 2 progenitor cell classes differently, elevating CFU-C levels, while markedly inhibiting CFU-M levels (Table 2).

Table 2

<table>
<thead>
<tr>
<th>Tumor</th>
<th>CFU-C</th>
<th>CFU-M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophage: J774</td>
<td>164</td>
<td>173</td>
</tr>
<tr>
<td>T-Lymphoma: EL4</td>
<td>90</td>
<td>89</td>
</tr>
<tr>
<td>B-Lymphoma: 70Z/2</td>
<td>164</td>
<td>30</td>
</tr>
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</table>

* Numbers in parentheses, range.

Therapeutic Effect of Mit-C and Restoration of Immune Responses in Mice Bearing 70Z/2, J774, or EL4 Tumors. Treatment of mice bearing 70Z/2 or J774 with Mit-C (Table 3) showed a dose-dependent restoration of in vitro CTL (Column 6) and anti-SRBC PFC (Column 7) responses which parallel the decrease in splenic tumor cells (Column 4) expressed as colony formation in soft agar. In the case of BD2F, mice bearing 70Z/2, a significant increase in MST was observed. Mit-C did not affect CTL or PFC responses in normal BD2F, or BALB/c mice until doses in excess of 0.3 mg/kg/day were administered. Although CTL responses appeared fully restored in Mit-C-treated, tumor-bearing mice, the number of splenic PFC did not exceed 50% of the PFC in controls. In contrast to the reduced in vitro PFC response in animals receiving Mit-C therapy, B-lymphocyte colony formation was unaffected by doses of up to 0.3 mg/kg/day (Column 8). A dose-dependent decrease in CFU-C and CFU-M levels was observed (Columns 9 and 10) at higher doses of this agent.

Mit-C reduced the tumor load in the spleens of EL4-bearing mice. However, C57BL/6 mice were very susceptible to the toxic effects of the drug, and all parameters measured were inhibited by doses of Mit-C as low as 0.1 mg/kg/day. Thus, no concentration of this drug was found which would reduce the tumor load and simultaneously restore immune function in this tumor-mouse strain combination.

Partial Restoration by ara-C of Immune Responsiveness without Increase in MST. Our previous report (46) indicated that ara-C reduced the total packed cell volume (ml) of EL4 ascites to zero at drug doses of 1 to 3 mg/kg/day. However, no significant increase in MST was seen. This chemical reduced the tumor load in spleens of mice bearing each of the 3 tumors (70Z/2 not shown) and partially restored immune parameters (Table 4). Tumor-free C57BL/6 mice did not show evidence of drug toxicity in the doses tested, and PFC and CTL responses were not significantly different from those of the controls. CTL responses in normal BALB/c mice were not affected by ara-C; however, PFC responses were abrogated at

Table 1

<table>
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<tr>
<th>Tumor</th>
<th>% specific *⁸⁶Cr release</th>
<th>PFC/10⁶ recovered cells</th>
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<tr>
<td></td>
<td>Tumor-free mice</td>
<td>Tumor-bearing mice</td>
</tr>
<tr>
<td></td>
<td>mice</td>
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<table>
<thead>
<tr>
<th>Tumor/mouse strain</th>
<th>Mit-C dose (mg/kg/day)</th>
<th>MST</th>
<th>Tumor cell colonies/spleen ( \times 10^{-3} )</th>
<th>Spleen cell recovery ( \times 10^{-4} )</th>
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**Table 3**

Effect of Mit-C therapy on immunological and hematopoietic capacities during progressive growth of 702/2, J774, and EL4 lymphoid tumors

<table>
<thead>
<tr>
<th>Immunological functions</th>
<th>Hematopoietic capacities</th>
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<tr>
<td>CTL Response % 111Cr release</td>
<td>CFU-B ( \times 10^{-9} )</td>
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<tr>
<td>PFC/108 recovered spleen cells</td>
<td>CFU-M AF</td>
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<td>LDH activity</td>
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<table>
<thead>
<tr>
<th>Tumor/mouse strain</th>
<th>CFU-B AF</th>
<th>CFU-M AF</th>
<th>CFU-C AF</th>
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<tbody>
<tr>
<td>702/BDxF1</td>
<td>932 ± 235</td>
<td>184 ± 56</td>
<td>9373 ± 472</td>
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<tr>
<td>J774/BALB/c Cr1</td>
<td>1032 ± 126</td>
<td>400 ± 63</td>
<td>4853 ± 166</td>
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<td>EL4/C57BL/6J</td>
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<td>189 ± 47</td>
<td>607 ± 161</td>
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* TB/TF, tumor-bearing mice/tumor-free mice.
Table 4
Effect of ara-C therapy on immunological and hematopoietic capacities during progressive growth of J774 and EL4

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<tr>
<th>Tumor</th>
<th>ara-C dose (mg/kg/day)</th>
<th>MST</th>
<th>Tumor cell colonies/spleen x 10^-3</th>
<th>Spleen cell recovery x 10^-3</th>
<th>CTL response % ^1Cr release</th>
<th>PFC/10^6 recovered spleen cells</th>
<th>CFU-B x 10^-3</th>
<th>CFU-M</th>
<th>CFU-C</th>
<th>LDH activity</th>
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<td>104</td>
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</table>

^1 TB/TF, tumor-bearing mice/tumor-free mice.
doses greater than 3 mg/kg/day. This reduction in PFC response occurred in the absence of demonstrable effects on CFU-B; however, hematopoietic progenitor cells were reduced at drug doses above 10 mg/kg/day.

**Successful Therapy of J774-bearing Mice by Poly(rI)-poly(rC) Therapy.** The most pronounced difference in responsiveness to therapy of the 3 lymphoid tumors was observed with the immunomodulator poly(rI)-poly(rC). Reduction in J774 splenic tumor load was readily apparent and correlated with an increase in MST. This occurred while B- and T-lymphocyte functions were restored (Table 5) in the absence of toxicity to hematopoietic precursor cells. Splenic B- and T-lymphocyte functions were also restored without toxicity of hematopoietic precursor cells (Table 5). In contrast, this agent did not have an antitumor effect and did not effect increase in MST of the immune functions in EL4-bearing mice; in fact, it was quite toxic to immunological responses in normal C57BL/6 mice. The CFU-B were not affected, however. Poly(rI)-poly(rC) enhanced the granulocyte-macrophage precursor levels 2- to 3-fold in spleens of C57BL/6 normal and tumor-bearing mice at high doses of the drug.

**Restoration of Immune Functions, but Differential Responses of Tumors to Act-D Therapy.** Act-D greatly increased the survival time of mice bearing 70Z/2 (Table 6). The increased MST correlated with reduced tumor load in the spleen and restoration of immune functions. Total nucleated cell recovery and hematopoietic function were not disturbed by administration of this agent to BD2F1 mice. Act-D was ineffective in increasing MST of mice bearing EL4 (not shown) and J774. However, immunological functions were restored in both strains of mice in parallel with the elimination of tumor cells from the spleen. This drug contrasts with ara-C, which reduced tumor load but failed to restore CTL or PFC responses in J774-bearing mice.

**LDH Virus Involvement in Tumor-induced Suppression.** Because LDH virus, harbored in tumor cell lines and endogenous to certain mouse strains, has been shown to be an immunosuppressive agent (30), we felt it necessary to monitor plasma LDH levels during chemotherapy. Replication of LDH virus in mice after tumor implantation could readily account for the observed suppression of immune responses in tumor-bearing mice. A fall in LDH levels paralleling therapy could argue that the immune dysfunction was a result of LDH virus propagation rather than of tumor progression. Tables 3 to 6 show that mouse stocks used in this study were free of LDH virus. Mice bearing 70Z/2 and J774 failed to show elevated LDH plasma levels, indicating that these tumors were free of this virus. C57BL/6 mice, although free of LDH virus in the absence of tumors, showed very high plasma LDH levels in EL4-bearing mice on the day of sacrifice (Tables 3 to 5). However, therapy with ara-C which allowed partial recovery of B- and T-cell responses did not significantly alter plasma LDH levels (Table 3), suggesting that LDH virus was not the cause of immunosuppression observed in these experiments.

**Suppression of Normal Spleen Cell Responses in Vitro by Syngeneic Tumor Cells.** Tables 3 to 6 demonstrate that spleens from tumor-bearing mice had significant tumor infiltration. It was possible that outgrowth of tumor cells during culture of tumor-bearing spleens accounted for the immune dysfunction we observed. To examine this possibility, known numbers of viable ascitic tumor cells were added to normal CTL and PFC induction cultures. Table 7 shows that the induction of CTL was readily suppressed in cultures of normal spleens when greater than 1,500 EL4 or 500 J774 cells were added to the generating cultures. This is equivalent to 650 EL4 or 135 J774 colony-forming units when corrected for plating efficiency. PFC responses were not suppressed until greater than 15,000 viable EL4 or J774 cells were added. 70Z/2 cells, in contrast, did not suppress in vitro development of either immune function at cell doses up to 50,000 cells (7,150 colony-forming units). Addition of in vitro-cultured tumor cells (data not shown) produced comparable suppression of spleen cell cultures, indicating that host cells contaminating the tumor ascites were not responsible for the observed suppression.

**DISCUSSION**

In the present study, abrogation of tumor-associated suppression of CTL and PFC responses accompanied therapy with tolerated doses of some drugs (summarized in Table 8). Significant reduction in splenic tumor load was observed in all tumor-drug combinations except in EL4 treated with poly(rI)-poly(rC). The cytoreductive effect of the drugs, however, was not always reflected in increased MST or restored immune responsiveness. The restoration of tumor-induced immune dysfunction by the chemotherapeutic agents listed did not correlate with changes in plasma LDH activity, indicating that LDH virus was not responsible for the suppressed immune functions in tumor-bearing animals.

**Effect of Tumor on Immune Function and Hematopoiesis.** 70Z/2 tumor cell colonies in spleens of untreated BD2F1 were in the range of 6.5 x 10³ colonies/10⁶ spleen cells. Tumor cell colonies in spleens of J774 or EL4 tumor-bearing mice were in the range of 640 J774 colonies/10⁶ spleen cells or 1160 EL4 colonies/10⁶ spleen cells, respectively. In each case, 5 x 10⁶ spleen cells were cultured in the CTL induction assay, and 2 x 10⁷ spleen cells were cultured in the anti-SRBC culture (Tables 3 to 6). We therefore calculated that PFC cultures containing spleen cells from untreated animals had 3200 70Z/2, 320 J774, and 580 EL4 tumor colony-forming cells, respectively, at the initiation of in vitro cultures. In all cases except 70Z/2, based on these calculations, suppression of PFC and CTL development by tumor cell outgrowth in untreated mice was expected. Mit-C and ara-C, at doses which did not affect control responses and reduced tumor below the predicted suppression level, did not completely restore B- and T-cell responses. We concluded from these calculations that tumor outgrowth in culture accounted for suppression in untreated controls but did not explain suppression after effective therapy (Table 3, Mit-C versus 70Z/2; Table 4, ara-C versus EL4).

The studies reported here do not allow a definite conclusion to be drawn as to the nature of the cell being suppressed in tumor-bearing mice. It is well recognized that PFC and CTL development require the collaboration of 3 lymphoid cell compartments. PFC development to SRBC requires B-cells, T-cells, and macrophages, while CTL development requires collaboration between 2 T-cell populations and macrophages (8, 20, 39). The tumor-induced immune dysfunction may reside in any one of these lymphoid cell compartments. Haba et al. (14) have shown that antibody responses to T-dependent antigens were suppressed in mice bearing Ehrlich ascites tumor, whereas the
Table 5

Effect of poly(r)-poly(rC) on immunological and hematopoietic capacities during progressive growth of J774 and EL4

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Poly(r)-poly(rC) dose (mg/kg/day)</th>
<th>MST</th>
<th>Tumor cell colonies/spleen x 10^-3</th>
<th>Spleen cell recovery x 10^-4</th>
<th>CTL response % $^{51}$Cr release</th>
<th>PFC/10^6 recovered spleen cells</th>
<th>CFU-B x 10^-3</th>
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* TB/TF, tumor-bearing mice/tumor-free mice.
### Table 6

**Effect of Act-D therapy on immunological and hematopoietic capacities during progressive growth of 70Z/2 and J774**

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<th>Tumor</th>
<th>Act-D dose (mg/kg/day)</th>
<th>MST</th>
<th>Tumor cell colonies/spleen x 10(^{-3})</th>
<th>Spleen cell recovery x 10(^{-4})</th>
<th>CTL response % (^{51}Cr) release</th>
<th>PFC/10(^6) recovered spleen cells</th>
<th>CFU-B x 10(^{-3})</th>
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\* TB/TF, tumor-bearing mice/tumor-free mice; ND, not done.
response to T-independent antigens was normal. The T-helper cells required for an antibody response to T-dependent antigens were markedly reduced in the tumor-bearing mice, suggesting that immune suppression in tumor-bearing mice may reside among the T-helper cell populations.

Claësson and Johnson (3) have reported that B-lymphoma and mammary carcinoma raise CFU-B and CFU-C levels in the spleens of tumor-bearing mice. A T-cell lymphoma raised splenic CFU-B levels but not those of CFU-C. In this study, no effect was seen on CFU-B by any of 3 tumors used. In agreement with the study of Claësson and Johnson, however, increased levels of splenic CFU-C were observed in mice bearing the B-lymphoma and macrophage tumors (Tables 3 to 6). The T-lymphoma did not influence CFU-C. Original to this study is the influence of tumor on CFU-M levels. J774 tumors raised splenic CFU-M levels in all experiments. This appears to be due to a potentiating activity produced by high concentrations of the tumor cell line. The T-lymphoma did not influence the CFU-M population, but surprisingly, the 70Z/2 B-lymphoma markedly depressed the megakaryocyte progenitor levels. No marked decrease in colony size was noted, however.

**Drug-Induced Restoration and Immune Function.** The restoration of *in vitro* immune responsiveness in spleen cells from tumor-bearing mice was achieved after therapy with chemicals, each of which affects tumor cells by a different mechanism(s) (4). Mit-C (Table 3) was very effective in reducing splenic tumor load in the 3 tumor-host combinations studied. Restoration of immunological responsiveness paralleled tumor reduction in mice bearing 70Z/2 or J774. This therapeutic effect resulted in a prolongation of survival in mice bearing 70Z/2. Total nucleated cell counts, CFU-B, or PFC responses were significantly reduced in tumor-free mice receiving Mit-C at doses greater than 1 mg/kg/day.

The failure of ara-C to overcome the tumor-dependent suppression of immune responses might be related to its low activity against tumor cells established in peripheral organs, as evidenced by significant tumor load in the spleens at termination of therapy. Hematopoietic progenitor cell levels and CFU-B-forming capacity in the spleen were not affected at the doses administered in this study. B-cell responses were severely impaired in untreated BALB/c mice. However, ara-C partially restored immune responses in tumor-bearing C57BL/6 mice. ara-C has been reported to preferentially reduce anti-SRBC responses in dogs receiving renal grafts (12), to reduce antibody responses to protein antigens in humans (29), and to reduce serum-blocking factors in tumor-bearing mice (15). It failed to prolong graft rejection (12) and did not reduce cell-mediated immune responses to mammary tumor antigens in C3H/HeJ mice (15).

The synthetic double-stranded polynucleotide poly(rI)-poly(rC) was very effective in reducing tumor load in J774-bearing mice. This was reflected in a 100% increase in MST and complete immunological reconstitution. Mice bearing 70Z/2 tumor were comparably protected by this agent. Doses higher than 3.0 mg/kg/day appear to be toxic for CTL responses in normal BALB/c mice. C57BL/6 mice were very sensitive to drug toxicity, in that immune responsiveness was drastically reduced at levels as low as 0.3 mg/kg/day. This agent differed from the other drugs tested in that CFU-C and CFU-M progenitor cell levels were measurably enhanced in normal mice receiving poly(rI)-poly(rC) (Table 5). The elevated progenitor cell levels can be accounted for by drug-induced splenic stimulatory factors (26, 42), rather than migration, since the regime of 5 mg/kg/day has been shown to induce marrow aplasia (25).
Therapy of Tumor-induced Splenic Immune Dysfunction

It has previously been reported that the effectiveness of poly(rI)-poly(rC) varies depending on the concentration and route administered. This agent has been reported to bind to tumor cells, thus enhancing its antigenicity (36). Cellular immune responses against syngeneic tumors have been reported to be significantly increased by pretreating tumor cells with this drug. This increase in antigenicity of the tumor cell could account for increased MST in J774- or 70Z/2-bearing mice. Immunopotentiating did not occur in the EL4-C57BL/6 tumor-host combination since no significant reduction in tumor load was observed. De Clercq et al. (5) reported that repeated doses of 5 mg/kg/day showed significant reduction of HT1080 fibrosarcoma in nude mice. This could possibly be due to activation by poly(rI)-poly(rC)-induced interferon of natural killer cells (7) known to be readily detectable in nude mice. Although interferon has been reported to reduce tumor load in several murine systems (13), it did not cause regression of the human HT1080 in nude mice. Levine et al. (23) were unable to demonstrate an antitumor response in patients receiving poly(rI)-poly(rC) therapy for solid tumors or leukemia.

Act-D, an agent active against several human tumors (4, 10), was only effective in prolonging survival of mice bearing 70Z/2. Although splenic tumor load was substantially reduced in mice bearing J774 or EL4, survival was not detectably increased. Anti-SRBC responses were readily reconstituted to control levels in each tumor system. CTL responses were concomitantly restored in J774- and EL4-bearing mice. This agent, administered in dosages of 0.01 mg/kg/day, did not significantly alter total nucleated cell recovery in the spleens or affect hematopoietic precursor or CFU-B. Drug-induced aplasia or preferential infiltration of tumor into bone marrow was not observed in treated tumor-bearing animals. We have established7 that ascites cells aspirated from J774-bearing mice receiving Act-D between 0.003 and 0.01 mg/kg/day are significantly reduced in their capacity to suppress in vitro immunological functions compared to untreated ascites cells. The in vitro growth rate and plating efficiency in soft agar were equal to untreated cells. These results suggest that Act-D affects the ability of the tumor to produce factor(s) responsible for suppression or that the lymphoid cell(s) responsive to the signal initiating suppression are effectively depleted by the drug. Alternatively, Act-D may alter tumor distribution in the host. These possibilities are currently being investigated.

This study determined that several tumor cell lines derived from cells involved in immune responses could preferentially inhibit host immune functions. In certain instances, the tumor-induced suppression could be reversed by chemotherapy. Reversal of immune dysfunction did not always coincide with increased survival. Conversely, in no tumor-host drug combination have we observed increased survival without intact host immune responsiveness. Our preliminary data indicate that a significant reduction in tumor load (in some cases with significantly increased MST) in the absence of measurable toxicity to hematopoietic function and with concomitant restoration of immune responsiveness in tumor-bearing hosts can be achieved. Such procedures are of importance for the preservation of immune function in the course of presently used chemotherapy regimes and as a basis for developing effective combination schedules which would not impair immunological status.

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