Altered Toxicity of 5-Fluorouracil following Treatment with Corynebacterium parvum

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ABSTRACT

Recent studies have demonstrated that systemic Corynebacterium parvum increases serum granulocyte-macrophage colony-stimulating factor and stimulates the proliferation of granulocyte-macrophage progenitor cells. It was hypothesized that more rapid cycling of granulocyte-macrophage progenitor cells would render the cells more sensitive to a cell cycle-specific chemotherapeutic agent. The colony-forming ability of bone marrow granulocyte-macrophage progenitor cells was assayed in vitro with soft agar cultures. C. parvum given before 5-fluorouracil in C57BL/6 mice increased the granulocyte-macrophage progenitor cell toxicity, the lymphopenic effect, and the lethality of 5-fluorouracil. When C. parvum was given after 5-fluorouracil, there was more rapid rebound of granulocyte counts to normal or supranormal levels.

INTRODUCTION

Preparations of killed Corynebacterium parvum can cause tumor regression in a variety of experimental tumor models, either administered by i.t. injection or administered systemically (6, 15, 19, 22, 27, 28, 31). C. parvum has also been shown to be an effective adjuvant, synergistic to the tumor inhibition of chemotherapy (4, 13, 14, 33).

Studies in our laboratory have demonstrated that systemic C. parvum has the ability to elevate rapidly the serum levels of CSF (16). CSF is a specific glycoprotein regulator of granulocyte-macrophage proliferation in vitro. It was hypothesized that elevated CSF levels might stimulate more rapid cycling of granulocyte-macrophage progenitor cells in vivo, render the cells more sensitive to a cell cycle-specific chemotherapeutic agent, and thus cause increased hematopoietic toxicity.

The present studies demonstrate that, in mice, pretreatment with high doses of C. parvum can increase the leukopenic effect and bone marrow toxicity as well as the lethality of 5-FU. Conversely when C. parvum was given after 5-FU, there was more rapid rebound of peripheral blood granulocyte counts to normal or supranormal levels.

MATERIALS AND METHODS

The mice used in these studies were C57BL/6 males 6 weeks to 6 months of age, supplied by The Jackson Laboratory, Bar Harbor, Maine. In the individual experiments, all mice were of the same age. The C. parvum was a dry-weight killed suspension (7 mg/ml; Lot 996-0) provided by Burroughs Wellcome and Co., Research Triangle Park, Raleigh, N. C. 5-FU was obtained from Roche Laboratories, Nutley, N. J. All injections of C. parvum and 5-FU were given i.p. WBC were obtained by lacerating the tail vein of nonanesthetized mice; counts were performed with a standard hemocytometer. Smears were stained for differential counts with Wright's stain.

Bone Marrow Colony-forming Cell Assay. A modification of the soft agar culture technique for hematopoietic cells, developed by Bradley and Metcalf (2), was used to assay bone marrow and spleen progenitor cells of granulocytes and macrophages (16). Briefly, the entire marrow contents of the shaft of 1 femur were expressed into McCoy's Medium 5A, pipetted to obtain a single-cell suspension, and then counted. Sufficient cells to give 50,000 cells/ml were added to the soft agar culture medium. One ml of cells with culture media was placed in a 35-mm plastic Petri dish with 0.025 ml of postendotoxin mouse serum known to have supramaximal colony-stimulating activity (24). After 7 days of incubation, colonies were scored with a dissecting microscope. Spleen cells were similarly cultured after single-cell suspensions had been obtained by teasing the spleen with sterile needles. The spleen cells were plated at a concentration of 100,000 cells/plate. Student's t test and $\chi^2$ test were used for analysis of data.

RESULTS

Effect of 5-FU and C. parvum on WBC. 5-FU in doses of 100 to 800 mg/kg caused a leukopenia that was dose dependent (Chart 1). Absolute counts of lymphocytes, granulocytes, monocytes, and eosinophils were depressed by these doses of 5-FU; however, the relative depression of lymphocytes by sublethal doses of 5-FU was less than that for the other cell forms. In a pilot experiment in which 1400 $\mu$g C. parvum i.p. was injected 24 hr prior to varying 5-FU doses, it appeared that the degree of WBC depression for all cell types was greater and the return toward normal at 10 days for nonlethal doses of 5-FU was less than that in mice given injections of 5-FU alone (Chart 1). When C. parvum was administered 24 hr after 5-FU, there was still a transient depression of WBC, but there was a more rapid recovery of granulocytes and monocytes toward normal or supranormal levels.

In further studies with additional groups of controls, C. parvum alone at a dose of 1400 $\mu$g i.p. injected into 25-g mice caused a decrease in total WBC at 7 days, which persisted through Day 10 and was due to a lymphopenia (Table 1). Absolute granulocyte and monocyte counts were elevated 2- to 3-fold at 3, 7, and 10 days. These findings were consistent with our previously reported studies of
leukocyte changes after C. parvum (16). These previous studies had also documented a significant decrease in lymphocytes, eosinophils, and total WBC 6 hr after C. parvum, with return to base-line values at 24 hr. In the studies shown in Table 1, when C. parvum was injected i.p. 24 hr before 200 mg 5-FU per kg were given, total WBC were significantly lower at Day 7 than they were after 200 mg 5-FU alone were given (p < 0.01) or when C. parvum was injected 24 hr after 5-FU was given (p < 0.05). At 10 days, rebound toward normal led to significantly higher total WBC in the group that received C. parvum 1 day after 5-FU than in the 5-FU-only group (p < 0.02), but WBC were not higher in the mice given injections of C. parvum 1 day before 5-FU. Absolute granulocyte counts were significantly lower than normal at 7 days for the group given 200 mg 5-FU per kg only (p < 0.001) as well as for the group given injections of C. parvum 1 day before 5-FU (p < 0.001). At Day 10, granulocytes were undetectable in the group given 5-FU only and in the group given C. parvum 1 day before 5-FU, but they had rebounded to normal levels in the group given injections of C. parvum 1 day after 5-FU. At Day 7, absolute monocyte counts were lower than normal in all 3 groups given injections of 5-FU and, at Day 10, remained low in the group given 5-FU only and in the group given injections of C. parvum 1 day before 5-FU, but they were no longer significantly decreased in the group given injections of C. parvum 1 day after 5-FU. These leukocyte counts are those of surviving mice, as indicated in Table 1, inasmuch as there were deaths in all groups receiving 200 mg 5-FU per kg.

Effect of C. parvum on Lethality of 5-FU. In the experiments shown in Table 1, mortality was greatest for the group given C. parvum 1 day before 5-FU and was significantly greater (p < 0.05) than the mortality for mice given injections of C. parvum 24 hr after 5-FU. Additional studies were carried out to confirm further the increased mortality of mice given injections of C. parvum 1 day before 5-FU. The representative experiment in Chart 2 indicates a pattern of decreased survival and earlier death when mice were given injections of C. parvum 24 hr before the higher doses of 5-FU. An injection of C. parvum 1 day after 5-FU did not appear either to increase or to decrease lethal toxicity of 5-
Table 1

Effect of *C. parvum* and 5-FU on peripheral WBC

<table>
<thead>
<tr>
<th>Type of blood count</th>
<th>Time after beginning of treatment (days)</th>
<th>Treatmentsa</th>
<th>p b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.9% NaCl solutionc before 0.9% NaCld (Group 1)</td>
<td>0.9% NaCl solutionc before 5-FUd (Group 2)</td>
<td><em>C. parvum</em>c before 0.9% NaCld (Group 3)</td>
</tr>
<tr>
<td>Total WBC</td>
<td>12,100 ± 584 (10)</td>
<td>6,700 ± 650 (10)</td>
<td>7,000 ± 350 (10)</td>
</tr>
<tr>
<td></td>
<td>3,118 ± 500 (10)</td>
<td>3,850 ± 480 (9)</td>
<td>5,800 ± 400 (10)</td>
</tr>
<tr>
<td></td>
<td>7,100 ± 470 (10)</td>
<td>8,100 ± 500 (10)</td>
<td>3,200 ± 1,270 (3)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>10,800 ± 350</td>
<td>4,850 ± 550</td>
<td>4,000 ± 260</td>
</tr>
<tr>
<td></td>
<td>3,10,500 ± 550</td>
<td>7,785 ± 500</td>
<td>3,350 ± 400</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>1,280 ± 272</td>
<td>792 ± 93</td>
<td>2,550 ± 150</td>
</tr>
<tr>
<td></td>
<td>7,1,000 ± 100</td>
<td>4.2 ± 4.2</td>
<td>2,720 ± 250</td>
</tr>
<tr>
<td></td>
<td>10,1,200 ± 130</td>
<td>3,200 ± 360</td>
<td>1,740 ± 860</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1,48 ± 25</td>
<td>56 ± 16</td>
<td>340 ± 49</td>
</tr>
<tr>
<td></td>
<td>7,168 ± 57</td>
<td>395 ± 69</td>
<td>20.4 ± 15.4</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0,162 ± 44</td>
<td>5.9 ± 32</td>
<td>129 ± 32</td>
</tr>
<tr>
<td></td>
<td>3,135 ± 42</td>
<td>7.1 ± 7.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>7,10.1 ± 10.1</td>
<td>7.9 ± 7.9</td>
<td>12.6 ± 12.6</td>
</tr>
</tbody>
</table>

a All injections were given i.p. The first injection was given on Day 0. The second injection was given on Day 1. *C. parvum* was given at a dose of 1.4 mg/mouse. 5-FU was given at a dose of 200 mg/kg. There were 10 mice/group.

b Two-tailed t test.

c Injected on Day -1.

d Injected on Day 0.

e Injected on Day +1.

f Mean ± S.E.

g Numbers in parentheses, number of mice surviving.

h NS, not significant.
parvum 7 days prior to 5-FU compared to that in mice given
the spleen (16), a study was done to determine whether
difference between the lethality of 5-FU in mice given C.
tolerate larger doses of 5-FU. There was no significant
difference in the number of granulocyte-macrophage colony-forming cells in the
spleen (16), a study was done to determine whether
there might be protection of the mice by these larger
numbers of progenitor cells and whether the mice might
tolerate larger doses of 5-FU. There was no significant
difference between the lethality of 5-FU in mice given C.
parvum 7 days prior to 5-FU compared to that in mice given
0.9% NaCl solution 7 days prior to 5-FU (Chart 4).

Effect of C. parvum and 5-FU on Bone Marrow Granulo-
cyte-Macrophage Progenitor Cells. Forty-eight hr after
1400 µg C. parvum, there was a significant reduction in the
total number of cells per femur, but there was an increase
in the number of granulocyte-macrophage colonies devel-
oping per plate of 50,000 bone marrow cells and an appar-
ent 50% increase in the total number of bone marrow
granulocyte-macrophage progenitor cells (Table 2). In a
separate experiment, C. parvum injected 1 day before 20
mg 5-FU per kg resulted in granulocyte-macrophage pro-
genitor cell counts that were less than one-half of the level
of the group given injections of 5-FU only (p < 0.001).
Assays were performed 24 hr after the 5-FU injections
(Table 2).

Cyclic Treatment with 5-FU and C. parvum. Since C.
parvum and chemotherapy frequently are administered in
repeated courses, an experiment was designed to deter-
mine the toxicity of a cyclic treatment course of 5-FU alone
versus C. parvum 1 day prior to 5-FU versus C. parvum 1
day after 5-FU. The treatment course was repeated every 14
days. Only 47% of the mice in the treatment group in which
1400 µg C. parvum preceded 40 mg 5-FU per kg by 24 hr
were alive after the second treatment cycle, whereas all
given cyclic 5-FU alone survived 10 treatment cycles
(Table 3). Cyclic 5-FU followed by C. parvum 24 hr later
became a lethal treatment program after 7 treatment cycles;
the 1 mouse that died after the second treatment cycle died
from a lacerated liver at the time of i.p. injection. Similar
results were obtained in a repeat experiment with an addi-
tional control group given repeated injections of 1400 µg
C. parvum. Repeated i.p. injections of C. parvum alone in
Experiments 2 and 3 (Table 3) showed a mortality rate
similar to that from the group given C. parvum injections
44 hr after 40 mg 5-FU per kg.

The serial WBC changes after repeated injections of C.
parvum and/or 5-FU in Experiments 1 and 3 (Table 3) were
examined at 7-day intervals. Injection of 0.9% NaCl solution
i.p. and repeated bleeding for WBC and differential counts
did not cause a significant change in these counts (Table
4). C. parvum injections on Days 1 and 14 resulted in a
leukopenia that was sustained through Day 28 and that was
due to a lymphocytopenia. There was a significant increase
in the number of granulocytes on Days 7 and 21, and
monocytes were increased at Days 14 and 21. Forty mg 5-
FU per kg alone (Table 5) caused minimal changes in total
WBC but a significant granulocytopenia and monocytopenia at Day 7. In the groups of mice in Experiment 3 (Table 3), which were given injections of 40 mg 5-FU per kg, there was a greater degree of leukopenia in the mice given injections of C. parvum 1 day before 5-FU compared to the mice given injections of C. parvum 1 day after 5-FU (Table 5). This leukopenia was most marked at the midpoint in the cycle (7 days after injection of 5-FU), i.e., Days 7, 21, and 35. The leukopenia at these time intervals was due to both a blocking of the granulocytosis induced usually by C. parvum and an increased lymphopenic effect when 5-FU was injected after C. parvum.

After the fifth treatment cycle and 2 days after injection of 5-FU, 5 of the surviving mice from each of the 3 groups in Experiment 1 (Table 3) along with 5 additional uninjected control mice were killed and assayed for spleen and liver weights and bone marrow and spleen colony-forming cells. Bone marrow granulocyte-macrophage colony-forming cells were markedly deficient in the group given cyclic 5-FU and in the group given cyclic C. parvum 1 day before 5-FU, whereas, in the group given cyclic C. parvum 1 day after 5-FU, bone marrow colony-forming cells were decreased to only 60% of normals (Table 6). Two weeks after the tenth treatment cycle, an additional 5 mice from each of the 3 cyclic treatment groups were assayed; the granulocyte-macrophage progenitor cells per femur of all 3 groups were within the normal range.

**DISCUSSION**

The results of these experiments indicate that a cell cycle-active chemotherapeutic agent (5-FU) increased lethality for normal mice when it was administered 24 hr after systemic administration of large doses of C. parvum. Fisher et al. (9) also found increased mortality in tumor-bearing animals when 5-FU and C. parvum were given repeatedly every 7 days, with C. parvum given 4 days after each dose of 5-FU. The doses of 5-FU (20 to 800 mg/kg or 60 to 2400 mg/sq m) used in these experiments in mice can be compared (18) to the usual daily doses in humans (12 mg/kg or 540 mg/sq m), administered daily for 3 to 5 days (21).

An increased sensitivity of the hematopoietic system to 5-FU injection after C. parvum treatment is indicated by greater depression of peripheral WBC and by depression of the bone marrow granulocyte-macrophage progenitor cells. Although the degree of granulocytopenia did not reach statistical significance in individual experiments, in 6 separate experiments granulocyte counts were lower at 3 and 7 days when C. parvum was injected 1 day before 5-FU compared to treatment with 5-FU alone. This is in contrast to the usual increases in granulocytes at 3 and 7 days, induced by C. parvum alone. Mortalities began to occur before Day 7 in the mice treated with C. parvum 1 day before 5-FU; therefore only surviving mice were available for determination of WBC and differentials. Quite possibly, the more leukopenic and granulocytopenic mice died. It is not apparent from these studies whether the increased lethality of 5-FU given after C. parvum was directly related to the increased hematopoietic toxicity only or whether there was increased toxicity in other cell systems as well. Certainly, the time of deaths is consistent with granulocytopenia and sepsis. Further studies in germ-free animals might help to elucidate the possible role of sepsis.

The changes reported in this study in the relative and absolute numbers of granulocyte and macrophage progenitor cells in the bone marrow after C. parvum were similar to those previously demonstrated by several investigators (5, 16, 30). Prior work in this laboratory has shown that similar doses of C. parvum in mice elevate CSF within 1 to 2 hr and that these elevations are sustained for more than 2 weeks (16). CSF is a leukopoietic factor(s) that is capable of causing clonal proliferation in soft agar of hematopoietic precursor cells to form granulocyte-macrophage colonies. Mice treated with C. parvum develop not only increased serum CSF but also an increase in splenic granulocyte-macrophage precursor cells at 7 days and an elevation of peripheral blood granulocytes and macrophages, which persists for up to 2 weeks. It has been demonstrated that hematopoietic progenitor cells are more sensitive to the in vivo administration of many chemotherapeutic agents when the cells are actively proliferating (3, 7). The increased sensitivity of granulocyte-macrophage progenitor cells to 5-FU after injection with C. parvum would indicate that these cells in the bone marrow are proliferating at an increased rate. It is proposed that through the CSF mechanism (and possible additional unknown mechanisms) C. parvum stimulates granulocyte-macrophage progenitor cells to proliferation at an increased rate and thereby renders increased percentages of the precursor cells subject to the cell cycle-specific killing effect of 5-FU. Thus, the increased production of peripheral blood granulocytes and monocytes in mice treated with C. parvum alone may come about by both expansion of the number of progenitor cells in the spleen and increase in the rate of production of end cells by the bone marrow.

Although increased sensitivity of rapidly proliferating
Table 3
Survival after cyclic 5-FU and cyclic 5-FU and C. parvum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group</th>
<th>Cycle 1</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
<th>Cycle 4</th>
<th>Cycle 5</th>
<th>Cycle 6</th>
<th>Cycle 7</th>
<th>Cycle 8</th>
<th>Cycle 9</th>
<th>Cycle 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU</td>
<td>1</td>
<td>30/30 (100)</td>
<td>30/30 (100)</td>
<td>30/30 (100)</td>
<td>30/30 (100)</td>
<td>30/30 (100)</td>
<td>25/25 (100)</td>
<td>25/25 (100)</td>
<td>25/25 (100)</td>
<td>25/25 (100)</td>
<td>25/25 (100)</td>
</tr>
<tr>
<td>C. parvum 1 day before 5-FU</td>
<td>2</td>
<td>29/30 (97)</td>
<td>14/30 (47)</td>
<td>14/30 (47)</td>
<td>14/30 (47)</td>
<td>14/30 (47)</td>
<td>9/25 (36)</td>
<td>8/25 (32)</td>
<td>8/25 (32)</td>
<td>7/25 (28)</td>
<td>6/25 (24)</td>
</tr>
<tr>
<td>C. parvum 1 day after 5-FU</td>
<td>3</td>
<td>30/30 (100)</td>
<td>30/30 (100)</td>
<td>29/30 (97)</td>
<td>29/30 (97)</td>
<td>29/30 (97)</td>
<td>24/25 (96)</td>
<td>21/25 (89)</td>
<td>18/25 (72)</td>
<td>14/25 (56)</td>
<td>11/25 (44)</td>
</tr>
<tr>
<td>Experiment 2 5-FU</td>
<td>4</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>C. parvum 1 day before 5-FU</td>
<td>5</td>
<td>10/10</td>
<td>7/10</td>
<td>7/10</td>
<td>7/10</td>
<td>7/10</td>
<td>7/10</td>
<td>5/10</td>
<td>4/10</td>
<td>4/10</td>
<td>4/10</td>
</tr>
<tr>
<td>C. parvum 1 day after 5-FU</td>
<td>6</td>
<td>10/10</td>
<td>1/10</td>
<td>1/10</td>
<td>1/10</td>
<td>1/10</td>
<td>1/10</td>
<td>1/10</td>
<td>1/10</td>
<td>1/10</td>
<td>1/10</td>
</tr>
<tr>
<td>0.9% NaCl solution</td>
<td>7</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
<td>7/10</td>
<td>7/10</td>
<td>5/10</td>
<td>4/10</td>
<td>4/10</td>
<td>4/10</td>
<td>4/10</td>
</tr>
</tbody>
</table>

- Treatment course was repeated every 14 days. The dose of 5-FU was 40 mg/kg, and the dose of C. parvum was 1400 µg/mouse.
- Numbers in parentheses, percentage of mice surviving.
- Five animals were removed from Groups 1, 2, and 3 for assay after Cycle 5.
- * is not significant.

Table 4
Effect of repeated injection of 1,400 µg C. parvum i.p. on WBC

<table>
<thead>
<tr>
<th>Type of count</th>
<th>Before treatment</th>
<th>First cycle</th>
<th>Second cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days after</td>
<td>14 days after</td>
<td>7 days after</td>
</tr>
<tr>
<td></td>
<td>first injection</td>
<td>first injection</td>
<td>second injection</td>
</tr>
<tr>
<td></td>
<td>0.9% NaCl</td>
<td>0.9% NaCl</td>
<td>0.9% NaCl</td>
</tr>
<tr>
<td></td>
<td>solution</td>
<td>solution</td>
<td>solution</td>
</tr>
<tr>
<td>Total WBC</td>
<td>13,000 ± 1,850*</td>
<td>10,500 ± 700</td>
<td>11,300 ± 800</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>11,100 ± 1,440*</td>
<td>9,550 ± 600</td>
<td>10,470 ± 980</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>1,520 ± 400</td>
<td>970 ± 130</td>
<td>660 ± 100</td>
</tr>
<tr>
<td>Monocytes</td>
<td>200 ± 40</td>
<td>100 ± 50</td>
<td>90 ± 50</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>120 ± 50</td>
<td>60 ± 30</td>
<td>95 ± 30</td>
</tr>
</tbody>
</table>

Mean ± S.E.; * 10 mice/group.
Table 5
Effect of 1,400 μg C. parvum i.p. and 5-FU on WBC

<table>
<thead>
<tr>
<th>Time after beginning of treatment (days)</th>
<th>Type of count</th>
<th>Cycle#</th>
<th>Pretreatment</th>
<th>Treatments§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5-FU alone</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Group 1)</td>
</tr>
<tr>
<td>0</td>
<td>Total WBC</td>
<td>20,420 ± 1,630</td>
<td></td>
<td>18,040 ± 1,200</td>
</tr>
<tr>
<td>7</td>
<td>Lymphocytes</td>
<td>18,440 ± 1,500</td>
<td></td>
<td>17,450 ± 1,200</td>
</tr>
<tr>
<td>14</td>
<td>Granulocytes</td>
<td>1,520 ± 250</td>
<td></td>
<td>1,540 ± 140</td>
</tr>
<tr>
<td>21</td>
<td>Monocytes</td>
<td>140 ± 50</td>
<td></td>
<td>120 ± 60</td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td>130 ± 50</td>
</tr>
<tr>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td>18 ± 13</td>
</tr>
<tr>
<td>42</td>
<td></td>
<td></td>
<td></td>
<td>60 ± 10</td>
</tr>
</tbody>
</table>

- Injection courses were repeated every 2 weeks.
- All groups were given injections of 5-FU (40 mg/kg) i.p. on Days 0, 14, and 28.
- C. parvum injected 24 hr before 5-FU.
- 5-FU given 24 hr before C. parvum.
- Mean ± S.E. for 10 mice.

For the group given 5-FU alone, p < 0.01 when the granulocyte count on Day 0 is compared with the count on Day 7; p is not significant when the Day 14 count is compared with the Day 21 count or when the Day 28 count is compared with the Day 35 count.

The mortality of 40 mg 5-FU per kg when given after C. parvum in a cyclic treatment program at 2-week intervals was particularly impressive (Table 3). When no C. parvum was given, repeated doses of 40 mg 5-FU per kg at 2-week intervals appeared to be well tolerated for 10 cycles. Fatalities in the group given C. parvum after 5-FU were similar in number to fatalities in the group given C. parvum alone.

The doses of C. parvum administered i.p. to mice in these experiments, as in most animal experiments, are much larger than the i.v. doses currently being used in humans (25, 32). The dose of 1400 μg i.p. used in mice corresponds to approximately 180 mg/sq m (18), compared to the usual doses in humans of 1 to 10 mg/sq m i.v. (20, 25). The doses used in these experiments were chosen because they have commonly been used in animal experiments and were found to be optimal i.p. doses. Further work is necessary to determine to what extent lower doses of C. parvum and i.v. C. parvum injected prior to chemotherapy will enhance chemotherapeutic toxicity. Preliminary work in our laboratory has demonstrated that in mice, doses of 1 to 4 mg C. parvum per sq m i.v. can cause elevation of serum CSF.

Since mice bearing syngeneic tumors have been shown to have transient alterations in the relative incidence of bone marrow macrophage progenitor cells (1, 11), it is possible that the interaction of C. parvum and 5-FU would have been somewhat different in tumor-bearing animals. However, serially transplanted tumors are very frequently contaminated with lactate dehydrogenase-elevating virus (26), which we have found to effect both the serum CSF activity (17) and the rate of proliferation of granulocyte-macrophage progenitor cells (R. Foster, Jr. and G. Keller, unpublished data). For the proper interpretation of the
Cyclic 5-FU versus 4. p < 0.01.

Treatment Normal 2 versus 3, not significant; Groups 2 versus 4, p < 0.05. Groups 3 performed 2 days after the fifth injection of 5-FU. There were 5 mice/group.

The present studies show that, when C. parvum is given either chemotherapeutic agents or ionizing radiation (20), it has been speculated that it might be of value in limited data in these experiments do suggest that, when C. parvum is given to mice after 5-FU in a cyclic treatment course, there are increased numbers of bone marrow granulocyte-macrophage progenitor cells, compared to mice treated with 5-FU only.

Fisher and Wolmark (10) have presented data that indicate that the macrophages produced by bone marrow granulocyte-macrophage progenitor cells after treatment with C. parvum have antitumor cytotoxicity. The increased production of cytotoxic macrophages after C. parvum may be an important element of the antitumor activity of C. parvum. If this is true, then it would be important that cytotoxic agents not be administered at a time when the granulocyte-macrophage progenitor cells were particularly sensitive.

In the planning and carrying out of trials in which immunostimulating agents such as C. parvum and chemotherapeutic agents are to be administered in combination, it will be necessary to assess carefully the effect that the timing of administration of these agents might have on hematopoietic toxicity as well as the possibility that the cytotoxic therapy might inhibit the potential effectiveness of the immunotherapeutic agent.

Table 6

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Normal mice</th>
<th>Cyclic 5-FU</th>
<th>Cyclic C. parvum 1 day before 5-FU</th>
<th>Cyclic C. parvum 1 day after 5-FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Colonies/50,000 bone marrow cells</td>
<td>34.5 ± 3.3abc</td>
<td>11.180 ± 1,520bcd</td>
<td>12.9 ± 3.9</td>
<td>14.4 ± 2.7</td>
</tr>
<tr>
<td>Colonies/femur</td>
<td>11,180 ± 1,520bcd</td>
<td>2,850 ± 900</td>
<td>2,071 ± 471</td>
<td>6,762 ± 1,111</td>
</tr>
</tbody>
</table>

a The injection cycle was repeated every 2 weeks. Assays were performed 2 days after the fifth injection of 5-FU. There were 5 mice/group.

b Mean ± S.E.

c Significance of difference of numbers of bone marrow colonies per femur by Student's t: Groups 1 versus 2, p < 0.01; Groups 2 versus 3, not significant; Groups 2 versus 4, p < 0.05. Groups 3 versus 4, p < 0.01.

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REFERENCES


Altered Toxicity of 5-Fluorouracil following Treatment with Corynebacterium parvum

Roger S. Foster, Jr.

Cancer Res 1978;38:850-858.