Role of Antitumor Immunity in Cyclophosphamide-induced Rejection of Subcutaneous Nonpalpable MOPC-315 Tumors

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ABSTRACT

Previously, we had reported that a single i.p. injection of 15 mg cyclophosphamide (CY) per kg cured most mice bearing large MOPC-315 tumors (20 to 25 mm; Day 12 to Day 16 tumors) but rarely cured mice bearing nonpalpable tumors (Day 4 tumors). Also, mice that were not cured if treated with CY, 15 mg/kg, when they had nonpalpable tumors could not be cured if treated again with CY, 15 mg/kg, when they had large tumors (14). Here, we show that CY therapy with 15 mg/kg at early stages of tumor growth did not lead to alteration in the biology of the tumor so as to cause an increased resistance to CY-tumoricidal effects, increased resistance to immune lysis, and/or decreased immunogenicity. Treatment of nonpalpable tumor bearers with CY, 15 mg/kg, prior to in vitro immunization of their spleen cells did not reduce the ability of the spleen cells to generate antitumor cytotoxicity in vitro. However, the level of antitumor cytotoxicity generated was lower than that exhibited by in vitro-immunized spleen cells from mice treated with CY, 15 mg/kg, when they had large tumors. With CY, 15 mg/kg, mice bearing nonpalpable tumors could be cured in two ways: (a) by treating a mouse bearing a nonpalpable tumor in the presence of a contralateral large tumor; (b) by adoptive transfer of immune spleen cells given 1 day post-CY therapy. Both procedures resulted in higher levels of antitumor immunity which was apparently responsible for the cure of the mice in cooperation with CY. Thus, the ineffectiveness of CY therapy with 15 mg/kg at early stages of tumor growth correlated with the presence of relatively low levels of host antitumor immunity.

INTRODUCTION

Antitumor immunity can influence the effectiveness of CY therapy for tumor bearers. An increase in the effectiveness of CY therapy was demonstrated in the presence of antitumor immunity developed by preimmunizing the tumor bearers (6, 17, 20) or by adoptively transferring immunity with lymphoid cells (9, 13). A decrease in the effectiveness of CY therapy was demonstrated when tumor bearers were immunosuppressed with antithymocyte serum (13, 22), X-irradiation (16, 22), or high doses of drug (17). Several mechanisms have been suggested for cooperation between chemotherapy and host antitumor immunity. The drug might do the following: (a) reduce the tumor burden to a level whereby existent host antitumor immunity can eliminate residual tumor cells (8); (b) slow tumor growth long enough to allow the development of potent host antitumor immunity (6); (c) render residual tumor cells more immunogenic, thus providing a superior stimulation for the development of host antitumor immunity (11, 12); (d) render residual tumor cells more susceptible to immune lysis (2); (e) eliminate suppressor cells, thus allowing the generation and/or expression of potent host antitumor immunity (14).

We have shown recently that a single i.p. injection of CY, 15 mg/kg, cured most mice bearing 20- to 25-mm s.c. tumors (Day 12 to Day 16 tumor bearers) (14) and that the curative effect was not due solely to the tumoricidal effects of the drug and required the presence of T-cell-dependent antitumor immunity (15). However, this dose of CY rarely cured mice bearing nonpalpable (Day 4) tumors. Moreover, mice that were not cured if treated with CY, 15 mg/kg, when they had nonpalpable (Day 4) tumors could not be cured if treated again with CY, 15 mg/kg, when they had 20-mm tumors (14). Thus, treatment of tumor bearers with CY at early stages of tumor growth prevented the curative effect of CY at later stages of tumor growth. Therefore, the work presented here was aimed at determining whether CY therapy at early stages of tumor growth leads to alteration in the biology of the tumor or decrease in host antitumor immunity. In addition, we evaluated whether mice bearing nonpalpable tumors can be cured by the same dose of CY in the presence of higher levels of antitumor immunity.

MATERIALS AND METHODS

Spleen Cell Suspensions. Cell suspensions were prepared from spleens of normal female BALB/c mice (8 to 12 weeks old; Laboratory Supply Co., Indianapolis, Ind.) or from CY-treated BALB/c mice bearing s.c. MOPC-315 tumors. In any individual experiment performed, the spleens used in each group were obtained from at least 3 but usually 5 to 7 mice. Single-cell suspensions were prepared by mechanical dissection between glass slides as described previously (19), and the viability as determined by trypan blue dye exclusion (0.4%) always exceeded 95%.

Tumors. The MOPC-315 plasmacytoma was maintained by serial s.c. inoculation in syngeneic BALB/c mice. Routinely, mice were inoculated with 3.5 x 10^6 viable MOPC-315 cells, a dose which is 1000 times greater than the minimal lethal tumor dose and kills the mice in 21 ± 1 days. Single-cell suspensions were prepared by mechanical dissection between glass slides as described previously (19), and the viability as determined by trypan blue dye exclusion (0.4%) always exceeded 85%. Two sources of MOPC-315 tumor cells were used, one from untreated tumor bearers ("parental line") and the other from tumor bearers that were treated with CY, 15 mg/kg, when they had nonpalpable (Day 4) tumors ("treated line").

Chemotherapy. CY (Cytoxan; Mead Johnson and Co., Evansville, Ind.) was administered by i.p. injection at a dose of 15 mg/kg at various times prior to in vitro immunization of the spleens.
Ind.) was dissolved in sterile distilled water (to a concentration of 20 mg/ml) and further diluted in minimum essential medium (Grand Island Biological Co., Grand Island, N. Y.). CY therapy given in these experiments consisted of a single i.p. injection of 15 mg/kg unless otherwise stated. Tumors were measured 3 times weekly with vernier calipers, and mice that had no detectable tumors 60 days after therapy were considered to be cured. Each experiment was repeated 2 to 4 times.

In Vitro Immunization. The method for generating antitumor cytotoxicity in lymphoid cells was described previously (5, 19). Briefly, responder spleen cells (75 x 10⁵) were cultured with mitomycin C-treated (50 µg/ml for 30 min) MOPC-315 stimulator tumor cells (2.5 x 10⁶) in Roswell Park Memorial Institute Tissue Culture Medium 1640 supplemented with 5% fetal calf serum, 1% nonessential amino acids, 50 units penicillin per ml, and 50 µg streptomycin per ml (Grand Island Biological Co.). The cultures were incubated at 37°C in 5% CO₂ in air for 5 days, the time required for the in vitro generation of optimal levels of antitumor cytotoxicity (19).

Antitumor Cytotoxicity Assay. Cell-mediated lysis was determined as we have described previously (19), utilizing the 3.5-hr in vitro ⁵¹Cr release assay (4). The percentage of specific ⁵¹Cr release was assessed by the following formula:

\[\% \text{ of specific } ⁵¹\text{Cr release} = \frac{T - C}{M - C} \times 100\]

where \(T\) is the percentage of release with test spleen cells, \(C\) is the percentage of spontaneous release by the target cells alone which ranged between 14 and 18%, and \(M\) is the percentage of maximal ⁵¹Cr release obtained by 3 cycles of freezing and thawing, which ranged between 78 and 81%. Each experiment was performed 2 to 4 times. We have observed, as have others (5, 18), that the level of antitumor cytotoxicity obtained with spleen cells of individual inbred mice immunized in vitro under identical conditions may vary substantially from one experiment to another. Still, the effect of treating tumor-bearing mice with CY was consistent. Therefore, in order to obtain a more accurate comparison of the levels of antitumor cytotoxicity exhibited by in vitro-immunized spleen cells at different times following CY therapy, the cytotoxicity index is provided. The cytotoxicity index was calculated as follows.

\[\% \text{ of specific } ⁵¹\text{Cr release with in vitro-immunized} \]

\[\frac{\text{spleen cells from tumor bearers}}{\text{spleen cells from normal mice}}\]

\[\text{and } \% \text{ of specific } ⁵¹\text{Cr release with in vitro-immunized} \]

\[\frac{\text{spleen cells from treated line}}{\text{spleen cells from parental line}}\]

The level of antitumor cytotoxicity is also provided, and it is presented as the mean ⁵¹Cr release of triplicate samples ± S.E. Variations in the ⁵¹Cr release between individual samples rarely exceeded 3% of the mean. All points that differed by 8% release or more from the level exhibited by in vitro-immunized normal spleen cells were judged to be significantly different by the Student t test (p = 0.05).

Antithymocyte Serum. ATS was obtained from Microbiological Associates (Walkersville, Md.) and stored at -20°C prior to use. Mice were given 3 i.p. injections of 0.25 ml each on Days 2, 4, and 7 post-CY therapy.

RESULTS

Comparison of the Effectiveness of CY Therapy for Mice Bearing Large MOPC-315 Tumors of the Parental Line or of the Treated Line. Experiments were performed to determine whether CY therapy at an early stage of tumor growth leads to alteration in the biology of the tumor or in host antitumor immunity. Rather than treating the tumor bearer again with CY when their tumor reached 20 mm, we transferred the treated tumor cells to new mice with an intact immune response, waited until the tumor reached 15 to 20 mm, and then treated the new host with CY, 15 mg/kg, i.e. The effectiveness of CY therapy for the mice bearing the treated line was compared to its effectiveness for mice bearing the same size tumors of the parental line (Table 1). This dose of CY had a similar curative effectiveness for mice bearing large tumors of the parental line or of the treated lines; 58 and 50% of the treated mice were cured, respectively. These results suggest that CY therapy with 15 mg/kg does not lead to alteration in the biology of the tumor.

Comparison of Tumor Cells from the Parental Line and from the Treated Line for Their Immunogenicity and Susceptibility to Immune Lysis. To test the suggestion that CY therapy at early stages of tumor growth does not alter the biology of the tumor, we evaluated the immunogenicity and susceptibility of treated tumor cells to immune lysis and compared them to those of the parental line. This was done by in vitro immunization of spleen cells from normal mice with MOPC-315 stimulator cells of the parental or treated tumor line and then testing against target cells of both tumor lines (Table 2). A similar level of antitumor cytotoxicity (~60% ⁵¹Cr release) against each tumor line was generated by in vitro immunization against either cell line. Thus, CY, 15 mg/kg, administered at early stages of tumor growth did not lead to alteration in the immunogenicity of the tumor cells or susceptibility to immune lysis.

Effect of CY Therapy of Nonpalpable Tumor Bearer on the Antitumor Potential of Their Spleen Cells on Various Days Posttherapy. Since CY therapy with 15 mg/kg at an early stage of tumor growth did not appear to alter the biology of the tumor, experiments were performed to evaluate its effect on host antitumor immunity. Mice bearing nonpalpable (Day 4) tumors were given a single i.p. injection of CY, 15 mg/kg, and on various days post-CY therapy, their spleen cells were examined for antitumor potential in vitro (Table 3). One day after CY therapy, the spleen cells had about the same antitumor potential as did normal spleen cells (cytotoxicity index, 1.08). On Day 2, a slight increase in antitumor potential occurred (cytotoxicity index, 1.52), and this gradually decreased back to the normal level on Day 7 (cytotoxicity index, 0.97) and below it by Day 10 (cytotoxicity index, 0.68) as tumor growth progressed. The pattern for the 0.9% NaCl solution-treated control showed a more gradual increase in antitumor potential until Day 7 (cytotoxicity index, 1.43) followed by a drastic decrease by Day 10 (cytotoxicity index, 0).

Comparison of the Level of Antitumor Cytotoxicity Exhibited by In Vitro-Immunized Spleen Cells That Were Obtained from Mice Bearing Nonpalpable or Large MOPC-315 Tumors at the Time of CY Therapy. We compared the antitumor potential of spleen cells derived from mice treated with CY when they had nonpalpable (Day 4) tumors to that of spleen cells derived from mice treated with CY when they had large tumors.

Table 1

<table>
<thead>
<tr>
<th>Tumor cells used for inoculation</th>
<th>Effect of therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro-immunized</td>
<td>Cured (%)</td>
</tr>
<tr>
<td>Parental line</td>
<td>22.3 ± 0.6</td>
</tr>
<tr>
<td>Treated line</td>
<td>19.3 ± 0.4</td>
</tr>
</tbody>
</table>

* Mean ± S.E.
tumors (Day 14 tumor bearers). This was done by inoculating mice with MOPC-315 tumors on 2 different days (10 days apart) so that the treatment of both groups of mice with CY was done on the same day. Subsequently, the in vitro immunization of their spleen cells was initiated on the same day, and the $^{51}$Cr release assay performed 5 days later was done for all groups of spleen cells on the same day (Table 4). We chose to present the results obtained on Day 2 post-CY therapy, since Day 2 post-CY therapy of nonpalpable tumor bearers is the time of the peak of antitumor potential of their spleen cells (as evident from Table 3), even though it is not the time of the peak of antitumor potential of spleen cells from large-size tumor bearers (14). The peak of antitumor potential of spleen cells from mice treated with CY when they had large tumors occurs at least several days later (14). Thus, by presenting the data comparing the level of antitumor potential of spleen cells from nonpalpable and large-tumor bearers 2 days posttherapy, we actually favor smaller differences between the groups than are obtained when the comparison is performed at later days posttherapy, at which time the antitumor potential of spleen cells from mice treated with CY when they had nonpalpable tumors decreases, whereas that from mice treated with CY when they had large tumors increases. In vitro-immunized spleen cells from mice treated with CY when they had nonpalpable tumors exhibited higher levels of antitumor cytotoxicity (42% $^{51}$Cr release) than did in vitro-immunized spleen cells from untreated nonpalpable tumor bearers or normal mice (31 and 26%, respectively). However, the level of cytotoxicity exhibited by in vitro-immunized spleen cells of CV-treated, nonpalpable tumor-bearing mice was much lower than that exhibited by in vitro-immunized spleen cells from mice that had large tumors at the time of therapy (e.g., 42% versus 83% and 15% versus 58% $^{51}$Cr release at an effector/target cell ratio of 100/1 and 20/1, respectively).

### Table 2

<table>
<thead>
<tr>
<th>Tumor size at time of CY therapy</th>
<th>Antitumor activity (% of specific $^{51}$Cr release) at following times posttherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (control) None</td>
<td>26.5 ± 1.3a</td>
</tr>
<tr>
<td>Nonpalpable</td>
<td>30.9 ± 1.2</td>
</tr>
<tr>
<td>Large</td>
<td>20.8 ± 1.4</td>
</tr>
<tr>
<td>Normal (control) 15 mg/kg</td>
<td>31.8 ± 1.3</td>
</tr>
<tr>
<td>Nonpalpable 15 mg/kg</td>
<td>42.1 ± 4.6</td>
</tr>
<tr>
<td>Large 15 mg/kg</td>
<td>83.1 ± 1.5</td>
</tr>
</tbody>
</table>

a Mean ± S.E.

b Mice bearing nonpalpable tumors (Day 4) at the time of therapy.

c Mice bearing 19.9 ± 0.4-mm (Day 13) tumors at the time of therapy.

d Mice bearing 31.9 ± 0.4-mm (Day 13) tumors at the time of therapy.

The Effectiveness of a Single i.p. Injection of CY (15 mg/kg) in Curing Mice Bearing Large MOPC-315 Tumors on One Side and Nonpalpable Tumors on the Other Side. Experiments were performed to determine whether nonpalpable tumors can be cured with CY, 15 mg/kg, in the presence of higher levels of antitumor cytotoxicity (Table 5). This was done by giving mice bearing 15-mm MOPC-315 tumors on one side a second inoculum of $3.5 \times 10^5$ tumor cells on the contralateral side, and 4 days later, when the large tumors were about 20 mm and the second tumors were nonpalpable, one-half of the mice was treated with CY, 15 mg/kg. Of the untreated mice, none was cured, and in 91% of these mice, the second inoculum resulted in progressively growing tumor nodules before the death of the mice; thus, concomitant immunity was not
observed. Of the treated mice, 91% were cured, i.e., the large tumors regressed, and the second inoculum did not result in tumor nodules. As in previous experiments, CY, 15 mg/kg, cured most mice bearing large tumors but not mice bearing nonpalpable tumors.

The Effectiveness of CY (15 mg/kg) for the Cure of Nonpalpable Tumor-bearing Mice in the Presence of Adoptively Transferred Immune Lymphoid Cells. To assess directly the potential contribution of antitumor immunity in CY-induced rejection of nonpalpable tumors, we evaluated whether adoptive transfer of immune spleen cells to mice bearing nonpalpable tumors 1 day after CY therapy will result in tumor eradication. Mice bearing large MOPC-315 tumors were treated with CY, 15 mg/kg, and 5 days later, when their tumors were regressing, the spleens were removed for use in adoptive transfer experiments. Mice bearing nonpalpable (Day 4) tumors were given a single i.p. injection of CY, 15 mg/kg, and 1 day later, one-half of the mice received an injection of 5 x 10^7 immune spleen cells (Table 6). All but one of the mice treated with CY alone died in about 33 days. On the other hand, most mice (75%) treated with CY in conjunction with transfer of immune lymphoid cells were cured, and the survival of the few mice (25%) that were not cured was extended to 50 days. Thus, in the presence of higher levels of immunity, administration of CY, 15 mg/kg, to mice bearing nonpalpable tumors resulted in the cure of most mice.

The Minimal Dose of CY Required for the Cure of BALB/c Mice Bearing Nonpalpable MOPC-315 Tumors in the Absence of Adoptive Immunity and the Role of T-cell-dependent Antitumor Immunity. Mice bearing nonpalpable (Day 4) tumors resulting from s.c. inoculation of 3.5 x 10^5 tumor cells were given a single i.p. injection of CY ranging from 5 to 300 mg/kg. With 15, 25, or 50 mg CY per kg, none of the mice was cured, although survival was prolonged. With 100 or 200 mg CY per kg, most mice were cured. With 300 mg/kg, all mice died of infection or cytotoxicity of the drug.

Next, we determined the role of T-cell-dependent antitumor immunity in the cure of mice treated with 100 or 200 mg CY per kg followed by 0.25 ml ATS on Days 2, 4, or 7 posttherapy (Table 7). This protocol of ATS treatment was shown to abolish mice with ATS (from 100 to 73 or 80%, respectively). Thus, mg CV per kg was reduced only slightly by treatment of the given a single i.p. injection of various doses of CV ranging from 5 to 300 mg/kg. With 15, 25, on 50 mg CV per kg, none of the mice were cured, and the survival of the few mice (25%) that were not cured was extended to 50 days. Thus, in the presence of higher levels of immunity, administration of CV, 15 mg/kg, to mice bearing nonpalpable tumors resulted in the cure of most mice.

Table 5

<table>
<thead>
<tr>
<th>Size of tumors</th>
<th>CY therapy</th>
<th>No. of mice showing tumor at the second site</th>
<th>Cured (%)</th>
<th>Cured/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpalpablea</td>
<td>15 mg/kg</td>
<td>82</td>
<td>10/12</td>
<td>10/12</td>
</tr>
<tr>
<td>Large</td>
<td>15 mg/kg</td>
<td>0/12</td>
<td>8/12</td>
<td>8/12</td>
</tr>
<tr>
<td>Large and nonpalpableb</td>
<td>15 mg/kg</td>
<td>0/12</td>
<td>8/12</td>
<td>8/12</td>
</tr>
<tr>
<td>Large and nonpalpablec</td>
<td>None</td>
<td>0/12</td>
<td>0/12</td>
<td>0/12</td>
</tr>
</tbody>
</table>

a Mice bearing nonpalpable (Day 4) tumors at the time of therapy.
b Mice bearing 20.5 ± 0.4-mm (Day 12) tumors at the time of therapy.
c Mice bearing 20.5 ± 0.4-mm (Day 12) tumors on one site and nonpalpable (Day 4) tumors on the contralateral site.

d Meant ± S.E.
e Immune spleen cells obtained from mice that are in the process of rejecting 18.8-mm MOPC-315 tumors following therapy with CY, 15 mg/kg, given 6 days earlier.

The Effectiveness of CY therapy with 15 mg/kg at early stages of tumor growth, since CY therapy with 15 mg CV per kg led to the cure of most mice. This demonstrated the cooperation between CY-tumoricidal effects and antitumor immunity in the eradication of nonpalpable MOPC-315 tumors. Although CY, 15 mg/kg, cured most mice bearing large tumors, this dose of drug was not effective in curing mice bearing large tumors that were treated previously with CY, 15 mg/kg, when they had nonpalpable tumors (14). This did not appear to be due to modification of the tumor cells by CY at early stages of tumor growth, since CY therapy with 15 mg CY per kg was curative for mice bearing large tumors derived by injection of the treated line into new mice. Moreover, tumor cells from the treated line were similar to tumor cells from the parental line in their immunogenicity as assessed by their ability

Table 6

<table>
<thead>
<tr>
<th>Treatment of nonpalpable tumor bearers</th>
<th>Cured (%)</th>
<th>Survival time of mice not cured (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>12/15</td>
</tr>
<tr>
<td>CY, 15 mg/kg</td>
<td>100</td>
<td>15/15</td>
</tr>
<tr>
<td>CY, 15 mg/kg, + ATSa</td>
<td>73</td>
<td>11/15</td>
</tr>
<tr>
<td>ATS</td>
<td>0</td>
<td>0/15</td>
</tr>
</tbody>
</table>

a One-fourth ml given i.p. on Days 2, 4, or 7 post-CY therapy.

The curative effect of the large dose of CY for mice bearing nonpalpable (Day 4) tumors is due primarily to the tumoricidal activity of CY.

DISCUSSION

Previously, we reported that a single i.p. injection of CY, 15 mg/kg, cured most mice bearing large (20 to 25 mm; Days 12 to 16) MOPC-315 tumors but rarely cured mice bearing nonpalpable (Day 4) tumors (14). Here, we show that the ineffectiveness of CY therapy with 15 mg/kg at early stages of tumor growth was apparently not due to the presence of tumor cells with increased resistance to CY-tumoricidal effects, increased resistance to immune lysis, and/or decreased immunogenicity. However, it correlated with the presence of relatively low levels of antitumor immunity. Transfer of "immune" spleen cells to mice bearing nonpalpable tumors 1 day post-CY therapy with 15 mg/kg led to the cure of most mice. This demonstrated the cooperation between CY-tumoricidal effects and antitumor immunity in the eradication of nonpalpable MOPC-315 tumors.
to stimulate the in vitro generation of antitumor cytotoxicity and their susceptibility to immune lysis by in vitro-immunized cells. Thus, it appears that no alteration in the biology of the tumor resulted as a result of CY therapy with 15 mg/kg at early stages of tumor growth. Consequently, the ineffectiveness of the second dose of CY in a 2-dose model [see above (14)] could not be attributed to a change in the biology of the tumor.

Although treatment of mice bearing nonpalpable MOPC-315 tumors with CY, 15 mg/kg, resulted in augmented levels of antitumor cytotoxicity upon in vitro immunization, these levels were much lower than the levels of cytotoxicity exhibited by in vitro-immunized spleen cells from mice that were treated with CY, 15 mg/kg, when they had large tumors. Whereas the level of antitumor immunity exhibited by CY-treated, nonpalpable MOPC-315 tumor bearers appeared not to be enough to eradicate the remaining tumor cells, the level of immunity exhibited by CY-treated, large tumor bearers was enough. When the contribution of antitumor immunity of nonpalpable tumor bearers for the eradication of tumors was increased, CY, 15 mg/kg, cured most mice.

Increase in the level of antitumor immunity exhibited by mice bearing Day 4 nonpalpable tumors was accomplished in 2 ways. (a) We took advantage of the observations that, although mice bearing large tumors do not exhibit concomitant immunity as evidenced by their inability to reject a second challenge with viable tumor cells, their spleen cells exhibit augmented antitumor potential following CY therapy. Indeed, mice bearing large tumors that received a second tumor inoculum 4 days prior to CY therapy with 15 mg/kg were cured. (b) We adoptively transferred cells from mice which were in the process of rejecting a large tumor (15) to mice bearing nonpalpable tumors 1 day after CY therapy with 15 mg/kg [at a stage when CY has long been cleared from the circulation (10) and when it would not kill the transferred cells (9)]. For such transferred cells to aid in CY-induced tumor eradication, they have to reach tumor foci and either mediate tumor cell destruction (13) or recruit host cells which will mediate tumor destruction (7, 25).

We have shown previously that, although CY, 15 mg/kg, did not lead to the cure of mice bearing Day 4 nonpalpable tumors resulting from the inoculation of 3.5 × 10^6 tumor cells, this dose led to the cure of most mice bearing 10- to 14-day nonpalpable tumors resulting from inoculation of 1 × 10^9 tumor cells. These results emphasized the importance of time interval between tumor inoculation and CY therapy. However, they did not rule out the possibility that, at early stages of tumor growth, the tumor cells differ from those at late stages of tumor growth (Day 4 versus Days 10 to 14). This possibility is minimized by the results presented in this paper, which demonstrate that the treated line does not differ from the parental line in susceptibility to CY-tumoricidal effects, immunogenicity, and/or susceptibility to immune lysis.

Other investigators have shown that CY therapy is more effective when administered to tumor bearers exhibiting antitumor immunity due to previous immunizations than to unimmunized counterparts (6, 17, 20). Here, we show that the effectiveness of CY therapy correlated with the in vitro antitumor potential of the spleen cells from CY-treated tumor bearers. However, it remains to be established whether the cells involved in the in vitro generation and/or expression of antitumor cytotoxicity are also mediating in vivo antitumor immunity and are required for the cooperation with CY-tumoricidal effects in tumor eradication.

At least 100 mg CY per kg were required to cure most mice bearing nonpalpable (Day 4) MOPC-315 tumors in the absence of added immunity. The curative effect of the large dose of CY for mice bearing nonpalpable tumors was due primarily to the tumoricidal activity of CY as evident from experiments in which treatment of the mice with ATS only slightly reduced the effectiveness of the therapy. On the other hand, we showed previously that the curative effect of the low dose (15 mg/kg) of CY for mice bearing large (20 to 25 mm; Days 12 to 16) tumors was due not only to CY-tumoricidal effects but also to T-cell-dependent antitumor immunity, as evidenced by experiments in which the curative effect of CY was abrogated by treatment of the mice with ATS (15).

Whereas administration of CY, 15 mg/kg, to mice bearing nonpalpable tumors in the presence of augmented antitumor immunity resulted in the cure of most mice, a dose of at least 100 mg/kg was required to cure such mice in the absence of augmented immunity. Thus, by increasing the immunity of the tumor bearers, it might be possible to cure a tumor bearer with a lower dose of drug than required otherwise. Although mice bearing nonpalpable MOPC-315 tumors can be cured by a single i.p. injection with 100 mg CY per kg, this might not be so with other tumors which are not as susceptible to CY-tumoricidal effects as plasmacytomas (21, 24). A higher dose of drug might be more tumoricidal (23), but it might also be more immunosuppressive (1, 8, 16), thereby reducing the potential contribution of host antitumor immunity for tumor eradication and also increasing the susceptibility of the host to infection. The dose of drug required for the cure of tumor bearers might be decreased if the antitumor immunity exhibited by the tumor bearers is increased. In other words, when antitumor immunity is "weak," a larger dose of drug might be required to reduce the tumor load to a level with which the immune system can cope than that required when "strong" antitumor immunity is present.

ACKNOWLEDGMENTS

We wish to acknowledge the expert technical assistance of Katherine Siessmann, whose efforts were invaluable in this work, and also thank the staff of the Word Processing Center at the University of Illinois Medical Center under the supervision of Bridget Archer for keyboarding the manuscript.

REFERENCES

Cooperation between Chemotherapy and Immunity


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