Mechanism of Antitumor Action of Pyrimidinones in the Treatment of B16 Melanoma and P388 Leukemia

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ABSTRACT

This study was undertaken in an attempt to understand the mechanism of antitumor action of pyrimidinones alone and in combination with cyclophosphamide (CY). Pyrimidinones such as 2-amino-5-bromo-6-(3-fluorophenyl)-4(3H)pyrimidinone (ABMFPP) were relatively nontoxic toward murine L1210 leukemia cell growth in vitro with the concentration of drug required for a 50% inhibition of cell growth being >50 μg/ml. In contrast, ABMFPP showed anti-B16 melanoma activity in vivo which was sensitive to X-irradiation of the hosts. These results collectively suggest that pyrimidinones may act differently from conventional cytotoxic antitumor agents. Multiple i.p. injections of ABMFPP (125 mg/kg/injection) significantly augmented the cytotoxicity of both natural killer cells and macrophages in peritoneal exudates. The augmentation of both effector cell populations was delayed, but was more pronounced when animals received a dose of CY (100 mg/kg) prior to ABMFPP injections. The combination of CY and ABMFPP also showed a synergistic anti-P388 leukemia effect which appeared to be related to the initial reduction of the tumor burden by CY and the marked augmentation of the cytotoxicity of both natural killer cells and macrophages by ABMFPP. The antitumor activity of ABMFPP against B16 melanoma was almost completely eliminated when animals received a dose of 400 rads X-irradiation 5 days prior to tumor inoculation or a dose of 200 rads X-irradiation followed by several injections of anti-asialo monosialoganglioside antibody. The administration of anti-asialo monosialoganglioside alone also markedly reduced the anti-B16 melanoma activity of ABMFPP. The magnitude of reduction of the antitumor effect of ABMFPP by radiation and/or anti-asialo monosialoganglioside antibody directly correlated with the inhibition of the ABMFPP-mediated augmentation of immune responses. These results strongly suggest that the antitumor effect of ABMFPP alone or in combination with CY is at least in part mediated through its augmentation of natural killer cell and/or macrophage activities.

INTRODUCTION

We have observed a marked synergistic antitumor activity against P388 leukemia in mice receiving a single i.p. injection of CY24 hr after tumor inoculation (106 cells/mouse) followed by multiple i.p. injections of a pyrimidinone such as ABMFPP. Eighty % of the animals receiving combination therapy were long-term survivors (>30 days) as compared to 20% survivors when the animals received CY alone (7). None of the pyrimidinones alone had any significant therapeutic effect against P388 leukemia, although some of them showed marginal activities against B16 melanoma. The synergistic effect was also observed with B16 melanoma when animals received the combination therapy (7).

This study was undertaken in an attempt to understand the mechanism of action of pyrimidinones when used alone in the treatment of B16 melanoma and when used in conjunction with CY in the treatment of P388 leukemia. ABMFPP was used throughout the study. Since several pyrimidinones have been shown to induce interferons (14,18) and markedly augment NK cells (10,12,16), the immunomodulatory effects of ABMFPP on the tumoricidal activities of NK cells and macrophages were studied.

MATERIALS AND METHODS

Agents and Vehicles. CY, purchased from Sigma Chemical Co., St. Louis, MO, was dissolved in sterile 0.9% NaCl solution (saline). Pyrimidinones, including ABPP and ABMFPP were made at The Upjohn Co., Kalamazoo, MI, using published procedures (18,19), and the structures are shown in Chart 1. These compounds were prepared in fine suspension with Sterile Vehicle 100, made for laboratory use at The Upjohn Co. prior to injection into the animals. Sterile Vehicle 100 is composed of 5 mg carboxymethylcellulose, 4 mg polysorbate 80, 9 mg sodium chloride, and 9 mg benzyl alcohol in 100 ml water. Anti-asialo-GM1 antibody was obtained from WakO Chemicals, Dallas, TX.

Animals and Tumors. Male [C57BL/6 × DBA/2 F1] mice and male [BALB/c × DBA/2 F1] mice were supplied by Frederick Cancer Research Facility, Frederick, MD. These mice were generally 5 to 6 weeks of age and weighed 18 to 22 g. Groups of 10 mice were housed in suspended metal cages and were given pelleted food and water ad libitum. The B16 melanoma was maintained by continuous s.c. passage in syngeneic female C57BL/6 mice. For the therapeutic experiments, BD2F1 mice received an i.p. injection of 1:20 tumor brei. P388 leukemia was maintained by continuous i.p. passage in syngeneic female DBA/2 mice. This tumor was inoculated i.p. in CD2F1 mice for the experiments.

Animal Experiments. For therapeutic evaluation, P388 leukemia tumor cells (106 cells/mouse) were inoculated i.p. on day 0. One day later, the animals received a single i.p. injection of CY. The pyrimidinone was injected i.p. starting on Day 2 and every 4 days thereafter for a total of 7 injections. The experiment was terminated on Day 30. For B16 melanoma, 0.5 ml of tumor brei diluted 1:20 was inoculated on Day 0, the pyrimidinone was administered i.p. starting on Day 2 and was given weekly thereafter for a total of 7 injections. The experiment was terminated on Day 60.

The therapeutic response was measured as the median day of death of 10 mice/group according to the procedure of Schabel et al. (13) in which the median life span was determined with the dying animals only. The long-term survivors, in this case 30-day survivors for P388 leukemia and 60-day survivors for B16 melanoma, were recorded separately. Percentage increase of life span (ILS) was calculated as:

$$\frac{\text{Median day of death of control group} - 1 \times 100}{\text{Median day of death of treated group}}$$
In Vitro Assay for Cytotoxicity of Either NK Cells or Macrophages.

Effector cells prepared from peritoneal exudates were adjusted to 5 × 10⁶ cells/ml and 3 serial 3-fold dilutions were made with RPMI 1640. Then, 0.1 ml of each cell suspension was added to triplicate wells of a 96-well V-bottomed microtiter plate (Dynatech Laboratories, Alexandria, VA). The corresponding labeled target cells (YAC-1 cells for assaying NK cell activity or P815 tumor cells for assaying macrophage activity) were adjusted to 5 × 10⁵ cells/ml (and 5 × 10⁶ sheep RBC/ml with the P815 cells), and 0.1 ml of the cell suspension was added to the appropriate wells. Thus, the final effector cell target cells ratios would be 100, 33, 11, and 3.7. The plates were covered and centrifuged at 200 × g for 6 min and then incubated at 37°C in a humidified atmosphere of 5% CO₂ and 95% air for 3 to 4 hr for assaying NK cell activity and 20 hr for assaying macrophage activity. After incubation, the plates were centrifuged again, and 0.15 ml of the supernatant was removed from each well, transferred to glass tubes, and counted in a Packard gamma scintillation counter.

The specific cytotoxicity of either NK cells or macrophages was calculated according to the following equation:

\[
\% \text{ of cytotoxicity} = \frac{\text{Test cpm} - \text{spontaneous release cpm}}{\text{Total cpm} - \text{spontaneous release cpm}} \times 100
\]

where test cpm, spontaneous release cpm, and total cpm represent the radioactivity of 0.15-ml aliquots of the supernatant from the effector and target cell mixture, the supernatant of target cells only, and resuspension of target cells, respectively. Each microtiter plate contained at least one triplicate set of wells for the determination of total radioactivity and spontaneous release of radioactivity.

The activity of NK cells or macrophages was expressed in lytic units per 10⁶ cells. A lytic unit is defined as the number of effector cells required to generate a given percentage of cytotoxicity of a given target cell in a given length of time. In this study, a 10% cytolytic activity against P815 tumor cells in 20 hr was used for measuring macrophage activity, and a 10 or 20% cytolytic activity against YAC-1 cells in 3 hr was used for measuring NK cell activity. The actual calculation of lytic units involves linear regression analysis of the plot of percentage of cytotoxicity against the logarithmic ratio of effector:target cells. In most experiments, lytic units/10⁶ cells were converted to lytic units per mouse by multiplying the number of peritoneal exudate cells obtained from the treated mice. Under these assay conditions, P815 target cells were resistant to NK-mediated lysis (either splenic effector cells or nonadherent peritoneal exudate cells) in the 20-hr macrophage assay. Conversely, the NK-sensitive YAC-1 target cells were not lysed by activated macrophages in the short 3- to 4-hr assays.

Inhibition of L1210 Cell Growth in Culture. The basal medium used for growing mouse leukemia L1210 cells was RPMI Medium 1634 (Associated Biomedic Systems, Inc., Buffalo, NY). Fetal calf serum (5%), sodium bicarbonate (0.075%), and a mixture of penicillin (100 µg/ml of medium) and streptomycin (50 µg/ml of medium) were added as supplements. A 0.25-ml aliquot of diluent or drug was pipetted into each culture tube. The experiment was then initiated by the addition of 4.75 ml of cells (5 × 10⁶ cells/ml), and the tubes were incubated at 37°C for 3 days. Cell number was determined with a Coulter Counter (Coulter Electronics, Hialeah, FL). The ID₉₀ value was obtained by plotting the logarithmic drug concentration against the corresponding percentage of inhibition of cell growth (8).

RESULTS

Inhibition of L1210 Growth. Two pyrimidinones were tested in a 3-day cell growth inhibition assay in vitro. Neither ABPP nor ABMFPP showed a marked inhibitory effect on L1210 cell growth with ID₉₀ values greater than 50 µg/ml, whereas Adriamycin and actinomycin D, 2 clinically useful cytotoxic antitumor agents,
yielded ID_{50} values of 0.018 and 0.0007 μg/ml, respectively, under the same assay conditions.

**Effect of Radiation on the Antitumor Activity of Pyrimidinones.** The effect of radiation on the antitumor activity of CY and/or ABMFPP against B16 melanoma was investigated. Mice received a dose of X-irradiation of 200 rads (120 rads/min) 9 days prior to the tumor inoculation. One day after tumor inoculation, animals received a single i.p. injection of CY (200 mg/kg). ABMFPP was administered 2 days after tumor inoculation and weekly thereafter for a total of 7 injections. The results (Table 1) indicate that the antitumor effect of CY was not significantly altered when mice were pretreated with radiation. The median day of death of CY-treated animals with or without pretreatment with radiation was 24 and 23 days, respectively. However, the median day of death of the animals treated with only ABMFPP was reduced from 22 to 17.5 days (p < 0.05) when animals received the pretreatment with X-irradiation.

**Effects of CY and/or Pyrimidinone on NK Cell and Macrophage Activity.** Tumor-free CD2F1 mice received a single dose of CY (100 mg/kg) on Day 1 and a total of 7 injections of ABMFPP on the days indicated by the arrows in Chart 2. Peritoneal exudates were harvested on days 5, 9, 13, 19, and 23, and effector cells were prepared for assaying both NK cell and macrophage activities. Chart 2 clearly indicates that neither CY nor Sterile Vehicle 100 significantly induced NK cell or macrophage cytotoxic activities. However, in animals that received a dose of ABMFPP (125 mg/kg/injection) on Day 2, the cytotoxicity of NK cells was augmented and 793 lytic units were recorded 3 days after ABMFPP administration (Day 5 of the experiment). Approximately 580 lytic units were still detected 3 days after the second injection of ABMFPP. NK cell activity diminished to the background value on Day 13 in spite of the continuous injection of ABMFPP.

The administration of CY 1 day prior to ABMFPP injection appeared to delay the augmentation of NK cell activity by ABMFPP by about 4 days (Chart 2). Only low level NK cell activity (approximately 130 lytic units) was detected on Day 5 and augmentation reached a maximum on Day 9. Surprisingly, the augmentation of NK cell activity by ABMFPP was further enhanced by CY on Day 9. A maximum cytotoxicity of 2018 lytic units was recorded when animals received the combination treatment as compared to only about 800 lytic units when animals received ABMFPP treatment alone. Again, the enhancement diminished to the background level on Day 19.

A similar phenomenon was observed with the macrophage assay. ABMFPP significantly induced macrophage cytotoxic activity which did not reach the maximum until Day 13 after animals had received 3 injections of ABMFPP (Chart 2). CY further enhanced the augmentation of macrophage activity by ABMFPP by approximately 33%, although CY by itself did not significantly induce macrophage activity. However, the administration of CY did not delay the augmentation of macrophage activity by ABMFPP as it did with NK cell activity.

**Antitumor Activity of CY and/or ABMFPP against P388 Leukemia.** The antitumor effects of CY and/or ABMFPP were carried out simultaneously with the immunological experiment described in the previous section. In this experiment, the dosage and schedule of CY and/or ABMFPP administration were the same as those used in the immunological experiment with the exception that the animals received an i.p. inoculation of P388 leukemia cells (10^6 cells/ml) on Day 0. The results, as summarized in Table 2, indicate that the combination therapy was superior with a 123% ILS as compared to 95% ILS when animals received a dose of CY alone. Two of 10 animals that received combination therapy were long-term survivors (>30 days). ABMFPP alone showed no significant activity against P388 leukemia in vivo.

**Effect of Radiation and/or Anti-Asialo-GM1 Antibody on the Augmentation of Immune Responses by ABMFPP.** The augmentation of NK cell and macrophage activity by ABMFPP in BDF1 mice that received a dose of X-irradiation 7 days prior to the ABMFPP administration was compared to the activity in animals that did not receive radiation treatment. In both cases, the mice received a dose of ABMFPP (125 mg/kg/injection) on Days 2, 8, and 15. The results (Chart 3) indicate that macrophage cytotoxicity was significantly reduced at all time points in mice treated with 200 rads X-irradiation, whereas NK cell activity was significantly reduced at this dose only when measured on Day 6. The augmentation of either NK cell or macrophage cytotoxicity was virtually eliminated when animals received a dose of 400 rads X-irradiation.

Anti-asialo-GM1 antibody was inhibitory to the augmentation of both NK cell and macrophage activities by ABMFPP (Chart 4). Furthermore, the augmentation of both NK cell and macrophage activity by ABMFPP was almost completely eliminated when animals received a dose of X-irradiation (200 rads) on Day 5 followed by the injection of anti-asialo-GM1 (50 μg/injection) on Days -2, 4, 10, and 16 (Chart 4).

**Effect of Radiation and/or Anti-Asialo-GM1 Antibody on the Anti-B16 Melanoma Activity of ABMFPP.** The antitumor activity of ABMFPP against B16 melanoma was carried out simultaneously with the immunological experiment described in the previous section. In this experiment, tumor cells were inoculated i.p. on Day 0, and ABMFPP was administered i.p. starting on Day 2 and schedule of CY and/or ABMFPP administration were the exception that the animals received an i.p. inoculation of P388 leukemia cells (10^6 cells/ml) on Day 0. The results, as summarized in Table 2, indicate that the combination therapy was superior with a 123% ILS as compared to 95% ILS when animals received a dose of CY alone. Two of 10 animals that received combination therapy were long-term survivors (>30 days). ABMFPP alone showed no significant activity against P388 leukemia in vivo.

**Effect of Radiation and/or Anti-Asialo-GM1 Antibody on the Anti-B16 Melanoma Activity of ABMFPP.** The antitumor activity of ABMFPP against B16 melanoma was carried out simultaneously with the immunological experiment described in the previous section. In this experiment, tumor cells were inoculated i.p. on Day 0, and ABMFPP was administered i.p. starting on Day 2 and weekly thereafter for a total of 7 injections.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CY (mg/kg/injection)</th>
<th>Pyrimidinone</th>
<th>Median death (day)</th>
<th>ILS (%)</th>
<th>40-day survivors</th>
<th>Median death (day)</th>
<th>ILS (%)</th>
<th>40-day survivors</th>
<th>p *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile Vehicle 100</td>
<td>0</td>
<td>0</td>
<td>16.5</td>
<td>0</td>
<td>0/10</td>
<td>15</td>
<td>0</td>
<td>1/10</td>
<td>NS</td>
</tr>
<tr>
<td>CY + Vehicle</td>
<td>200</td>
<td>0</td>
<td>23</td>
<td>39</td>
<td>1/10</td>
<td>24</td>
<td>60</td>
<td>0/10</td>
<td>NS</td>
</tr>
<tr>
<td>ABMFPP</td>
<td>0</td>
<td>125</td>
<td>22</td>
<td>33</td>
<td>0/10</td>
<td>17.5</td>
<td>17</td>
<td>0/10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CY + ABMFPP</td>
<td>200</td>
<td>125</td>
<td>29</td>
<td>76</td>
<td>1/10</td>
<td>26</td>
<td>73</td>
<td>0/10</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Significance of the difference of median day of death from the corresponding nonirradiated group are determined by Student’s t test. NS, not significant, p > 0.05.

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Preface: It seems like the document contains scientific information on the action of pyrimidinones, specifically regarding their effects on macrophage and NK cell activity in mouse peritoneal exudates. The text discusses the administration of CY and ABMFPP, observing their effects on NK cell and macrophage activity in mice. It mentions the effects of CY and/or ABMFPP on antitumor activity in combination with radiation and anti-asialo-GM1 antibody. The discussion about the mechanism of pyrimidinone action is also included, comparing them to conventional antitumor agents and exploring their potential immune-modulating properties.

**DISCUSSION**

In attempts to understand the mechanism of the antitumor action of pyrimidinones, the general behavior of this class of compounds was compared to that of some conventional antitumor agents. The cytotoxic effect of pyrimidinones was studied initially, and it was found that ABPP and ABMFPP were at least 3000 times less cytotoxic against L1210 leukemia cell growth in culture when compared to Adriamycin or actinomycin D. These results imply that the antitumor activity of pyrimidinones may be mediated through some mechanisms other than those exhibited by cytotoxic antitumor agents.

This possibility was further explored by probing the radiation effect on the antitumor activity of pyrimidinones, in this case ABMFPP. When animals received a dose of X-irradiation (200 rads) 9 days prior to tumor inoculation, the anti-B16 melanoma activity of CY, another cytotoxic antitumor drug, was not markedly altered, but the antitumor activity of ABMFPP was significantly reduced (Table 1). Again, these results suggest that the mechanism of antitumor action of pyrimidinones is different from that of cytotoxic antitumor agents and is apparently dependent upon the same irradiation-sensitive components of the hosts.

Pyrimidinones, particularly ABPP, have been reported to have the ability to modulate a variety of immune responses including the enhancement of both in vitro and in vivo cytotoxicity of NK cells (11, 12, 16) and macrophages (14) as well as in vivo antibody production in nonimmunized and immunized mice (3). They have also been shown to be potent interferon inducers (5, 14). However, the direct comparison between the aforementioned biological effects of pyrimidinones and their antitumor effects had not been attempted previously.

Similar to the results reported previously (7), the antitumor effect of ABMFPP in combination with CY was superior (Table 2). A parallel experiment was carried out using tumor-free mice that received CY and/or ABMFPP treatment according to the

**Table 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CY (mg/kg/ injection)</th>
<th>ABMFPP</th>
<th>Median death (day)</th>
<th>ILS (%)</th>
<th>30-day survivors</th>
<th>Maximum activity induced (10% cytolytic activity/mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile Vehicle</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td>CY + Vehicle</td>
<td>100</td>
<td>0</td>
<td>21.5</td>
<td>95</td>
<td>0/10</td>
<td>88 (Day 9)</td>
</tr>
<tr>
<td>ABMFPP</td>
<td>0</td>
<td>125</td>
<td>12</td>
<td>9</td>
<td>0/10</td>
<td>793 (Day 9)</td>
</tr>
<tr>
<td>CY + ABMFPP</td>
<td>100</td>
<td>125</td>
<td>24.5</td>
<td>123</td>
<td>2/10</td>
<td>2018 (Day 9)</td>
</tr>
</tbody>
</table>

Tumor (approximately, 10^6 cells/mouse) was inoculated (i.p.) on day 0, and CY was injected (i.p.) on Day 1. ABMFPP was injected (i.p.) on Day 2 and every 4 days thereafter for a total of 7 injections.

Estimated from a titration curve constructed by Schabel et al. (13) based on a P388 leukemia generation time in animals of 16 hr.
We found that ABMFPP, like some other pyrimidinones (11, 16), significantly augmented the cytotoxicity of NK cells and macrophages from peritoneal exudates against their respective target tumor cells (Chart 2). The duration of the immunomodulating effects of ABMFPP was approximately 1 week. It is of interest to see that the responses of both NK cells and macrophages become hyporeactive after 5 injections of ABMFPP alone or in combination with CY treatment (Chart 2), since this phenomenon may be related to the anti-P388 leukemia activity of ABMFPP in combination with CY reported previously (7). In that study, we had showed that the synergistic antitumor effects were compatible whether animals received a total of 5, 6, or 7 injections of ABMFPP.

Although the administration of a single dose of CY prior to multiple injections of ABMFPP caused an approximate 4-day delay in the augmentation of NK cell activity by ABMFPP, CY not only did not inhibit the overall augmentation of NK cell and macrophage responses by ABMFPP but markedly enhanced them (Chart 2). There are at least 2 possible explanations for this observation. One is that CY has been reported to augment immunity by depleting suppressor T-cells and suppressor monocytes (2, 17). Whether this is related to its enhancement of the induction of cytotoxic effector cell responses by ABMFPP remains to be determined. The other possibility is that the immunomodulating effect of ABMFPP was overshot after overcoming the immunosuppressive pressure generated by the initial CY administration.

Table 2 illustrates the relationship between the immunomodulating effects of ABMFPP and the antitumor activity exhibited by the pyrimidinone alone or in combination with CY. Because the median day of death of animals demonstrated by some of us (7) and by Schabel et al. (13) was directly related to the number of tumor cells inoculated, the therapeutic effect could be estimated in terms of the tumor load reduction through a titration curve constructed by Schabel et al. (13) after 180 determinations. When animals received no CY treatment, they would carry approximately $8 \times 10^6$ cells/mouse (Table 2) 2 days after tumor inoculation (10$^6$ cells/mouse, initially). The estimated tumor load was derived from a generation time of about 16 hr obtained by determining the rate of tumor cell growth in the mouse peritoneal cavity (data not presented). Whereas in animals that received a single dose of CY (100 mg/kg) on Day 1, the median day of death increased from 11 days (untreated tumor-bearing animals)
to 21.5 days, which could be translated into the equivalent of approximately 75 cells remaining after receiving the CY treatment on Day 1 and approximately 200 tumor cells on Day 2 prior to the start of ABMFPP injections. No matter how and when the immunologically mediated killing of tumor cells occurred, the ratio of effector cells (in this case expressed as maximum lytic units of stimulated NK cells and macrophages): tumor cells in the peritoneal cavity would be about 90,000 times more favorable when animals received the combination therapy of CY and ABMFPP than when animals received ABMFPP treatment alone. Two of 10 animals receiving combination therapy were long-term (>30 days) survivors (Table 2). Although the message generated from this exercise, namely, that the antitumor activity of ABMFPP may be mediated in part through its immunomodulating effects and that its effectiveness is heavily dependent upon the tumor burden (1, 7, 9) appeared to be reasonable, a more direct proof is desirable.

Since some pyrimidinones, including ABMFPP, showed small but significant antitumor activity (from 25 to 50% ILS) against B16 melanoma when given alone, the anti-B16 melanoma activity of ABMFPP was compared in mice with and without X-irradiation treatment prior to ABMFPP administration. The results (Table 3) clearly indicate that radiation reduced the antitumor effect of ABMFPP in a dose-dependent manner. As discussed previously (7, 13), the life span of an experimental animal was directly related to the tumor load carried by that animal. The reduction of the percentage of ILS by radiation can be readily translated to percentage of inhibition of ABMFPP-mediated tumor reduction. For instance, 80% of the antitumor activity of ABMFPP was inhibited (p < 0.05) when animals were pretreated with a dose of 400 rads X-irradiation.

A parallel experiment was carried out with tumor-free animals according to the same experimental protocol used in the therapeutic experiment. The results (Table 3) show that radiation also inhibited the augmentation of NK cell and macrophage activities in a dose-dependent manner. Reduction of macrophage cytotoxicity by irradiation may reflect T-lymphocyte depletion resulting in lower lymphokine production and consequently less macrophage activation. The magnitudes of inhibition of the augmentation of immune responses mediated by ABMFPP by radiation were more or less equal to those of reduction of antitumor activity of ABMFPP. Hence, we have demonstrated here for the first time the direct relationship between antitumor activity of pyrimidinones and their immunomodulating effects. Results similar to those from the radiation experiment were obtained with anti-asialo-GM1 antibody which has been reported to inhibit selectively NK cell activity (6). However, this antibody does inhibit activated macrophages but not resident or elicited macrophages.4 In our hands, anti-asialo-GM1 inhibited the ABMFPP-mediated augmentation of NK cell and macrophage activities more or less equally (Chart 4). Although the selective effect of anti-asialo-GM1 requires further and closer scrutiny, the overall results indicate that when the pyrimidinone-mediated augmentation of cell-mediated immune responses was inhibited, so was its antitumor activity.

All in all, our results clearly indicate that the antitumor activity exhibited by ABMFPP and perhaps some other pyrimidinones are at least in part mediated through their augmentation of both NK cell and macrophage activities. Whether the induction of interferon by pyrimidinones (15) is also important to their antitumor and/or immunomodulating activities remains to be determined. Although we are not sure how these immune effector cells precisely work to eradicate the tumor population, their activities are magnified and become more significant when the tumor burden is small (Table 2; Ref. 7). Therefore, it is more beneficial to use pyrimidinone in conjunction with other therapeutic modalities in the treatment of experimental or clinical tumors (7, 9).

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* H. Holden, personal communication.


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