Inhibition and Enhancement of Friend Leukemia Virus by Pyran Copolymer

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SUMMARY

Inhibition or enhancement of Friend leukemia virus disease could be produced by treatment of mice with the immunopotentiator, pyran copolymer. The result depended on the route of inoculation of the drug. Prophylactic administration of the drug i.p. retarded splenomegaly, reduced splenic foci, and increased survival time of mice infected with Friend leukemia virus. Conversely, when the same dose and regimen of pyran was administered i.v., splenomegaly was enhanced, splenic foci were increased, and survival time was decreased. Histopathological examination of the spleens of mice revealed that i.p. pyran administration caused a marked increase in the splenic marginal zone with some increase in erythropoiesis in the red pulp, while i.v. pyran administration did not markedly change the splenic marginal zone but caused an early and sustained increase in erythropoiesis in the red pulp.

INTRODUCTION

Treatment of mice with the synthetic polyanionic immunopotentiator, pyran (divinyl ether maleic anhydride copolymer), has been shown to inhibit leukemogenesis induced by murine leukemia viruses (4, 9, 18) and to have antitumor virus and antitumor activity in several solid tumor systems (10, 14).

Although pyran can be an effective antitumor agent, like other immunopotentiators such as poly I:C, pyran has been shown to enhance murine sarcoma virus-induced tumor growth under certain conditions (8). The relationship between poly I:C and mouse sarcoma virus tumor induction is highly complex. The outcome of either reduction or enhancement of tumor formation depends on multiple factors including the age of mouse, strain of mouse, dose and time of administration of poly I:C, number of and site of injections of poly I:C, and dose of virus (5, 8). In addition, poly I:C and a double-stranded RNA of fungal origin have been demonstrated to increase severity of FLV disease depending on the time of treatment, amount of drug given, and frequency of dosage (12, 17). The mechanisms involved in these protective or enhancing effects of immunopotentiators are not well understood, although modulation of the antitumor immunological response has been cited as a possible factor.

Enhancement of the FLV disease has also been produced by leukogenenol (C_{18}H_{25}N_{0.8}), a leukocytosis-inducing factor extracted from bovine liver. Elliott et al. (7) suggested that the enhanced leukemogenesis resulted from an increased stem cell pool. Accelerated leukemogenesis of Rauscher virus has also been reported to occur with treatment of mice with the immunopotentiator, bovine serum albumin, in complete Freund's adjuvant, or with biological agents such as lactic dehydrogenase virus which also apparently act to enlarge target cell pools. Not only has enhancement been demonstrated in leukemia and sarcoma virus systems, but also tumors were enhanced in hamsters bearing transplantable adenovirus type 12 tumor cells and treated with complete Freund's adjuvant (1). In this paper, we report that by merely changing the route of inoculation of the immunopotentiator, pyran, a pronounced protective effect changes to a potentiation of FLV leukemogenesis. Histopathological study revealed that the enhancement was correlated with a marked increase in splenic erythropoiesis, while protection was correlated with enlargement of the splenic marginal zone.

MATERIALS AND METHODS

Pyran Copolymer. Pyran (XA124-177) was received courtesy of Dr. D. Breslow, Hercules, Inc., Wilmington, Del. (3). Prior to use the drug was dissolved in 0.15 M NaCl, the pH was adjusted to 7.0 to 7.2 with 0.15 N NaOH, and the drug was kept at 4° for up to 2 weeks.

Mice. Adult BALB/c male mice weighing 18 to 23 g were used.

Virus. FLV was obtained courtesy of Dr. R. Eckner, Roswell Park Memorial Institute, Buffalo, N. Y. The virus preparation contained both SFFV and LLV components and was NB tropic (6). Virus stocks were prepared from spleens taken from mice that were infected 10 days previously with FLV. Spleens were expressed through a mesh wire screen and homogenized in a Potter-Elvehjem Teflon tissue grinder in Hanks' balanced salt solution. A 10% suspension (w/v) was prepared in Hanks' balanced salt solution.

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1 This research was supported by NIH Grant CA 10537 and AI 00382. This paper was presented in part at the Tenth Meeting of the Reticuloendothelial Society (20).
2 Recipient of USPHS Research Career Development Award AI 70863.
3 The abbreviations used are: poly I:C, polyriboinosinic:cytidylic acid; FLV, Friend leukemia virus; SFFV, spleen focus-forming virus; LLV, lymphatic leukemia virus; MST, mean survival time.
Groups of mice were treated prophylactically with pyran when the FLY infection was not initiated for 4 weeks or by the prolonged prophylactic i.p. administration of pyran doses of the drug. Similar differences in effectiveness were (125 mg/kg i.p.) on Day —1 before infection or with 25

RESULTS

Protection against FLY by i.p. Administration of Pyran. Groups of mice were treated prophylactically with pyran (125 mg/kg i.p.) on Day —1 before infection or with 25 mg/kg i.p. for 5 days before infection (total of 125 mg/kg), or were treated therapeutically with pyran (25 mg/kg i.p.) for 5 days after FLY infection. Administration of pyran i.p., either before or after infection of mice with FLY, significantly inhibited development of splenomegaly and splenic foci (Table 1; Fig. 1). However, prophylactic treatment with a single dose of pyran or therapeutic treatment with pyran was not as effective as was prolonged prophylaxis with low doses of the drug. Similar differences in effectiveness were obtained when the growth of the SFFV component was measured.

The protection that was produced by prolonged prophylaxis was still present if infection with FLY was delayed for almost 3 weeks (Table 1). However, there was no protection when the FLY infection was not initiated for 4 weeks or more after prophylaxis.

The suppression of FLY splenomegaly that was produced by the prolonged prophylactic i.p. administration of pyran prior to i.p. FLY infection was maintained in some mice for as long as 100 days after virus challenge. FLY alone produced a splenomegaly of 1918 ± 186 mg by Day 18, while FLY-infected, pyran-treated mice developed a splenomegaly of only 431 ± 39 mg. When the survivors (5 of 10 mice) from this latter group were killed on Day 100, their spleen weight was 338 ± 50 mg, which was similar to that observed on Day 18 postinfection. The suppression of the virus-induced splenomegaly also resulted in an increase in MST. The MST of mice infected with FLY was 49 ± 5.5 days and that of pyran-treated, FLY-infected animals was 114 ± 15 days. No deaths occurred in mice that were given injections of pyran alone or in control mice given 0.15 M NaCl solution.

In contrast to the marked protection with i.p. administration of pyran against i.p. infection with FLY, the same drug regimen did not affect FLY-induced splenomegaly when the virus challenge was i.v. The splenomegaly of FLY-infected or pyran-FLY-infected mice was approximately the same (Table 2).

Enhancement of FLV by i.v. Administration of Pyran. The above results indicated that interactions between the drug and the virus within the peritoneal cavity might be important in protection against FLV leukemogenesis. Thus, FLY was also given i.v. with i.v. pyran which should enhance drug-virus interactions in the target organ, the spleen. Therefore, pyran was inoculated i.v. on Day —5 through —1 before i.v. inoculation of FLY (Table 2). Spleen weight was significantly enhanced by the i.v. inoculation of both pyran and FLY (12.82 ± 0.52% body weight) as compared to i.v. inoculation of FLY alone (4.68 ± 0.59% body weight). In order to determine whether this enhancement was due to the local interaction of i.v. pyran and i.v. FLY or due to the different route of administration of pyran, pyran was inoculated i.v. and FLY was inoculated i.p. The disease process was still enhanced (Table 2; Fig. 1). Splenic foci were correspondingly increased whenever enhanced splenomegaly occurred. A decrease in survival time also occurred in mice in which FLY-induced splenomegaly was enhanced. A MST of 45 ± 2.9 days compared to a MST of 53 ± 2.0 days occurred in mice infected with FLY. In mice given pyran (12.5 mg/kg i.p.) against FLY i.p. infection, survival was prolonged (MST, 66 ± 2.0 days) (Chart 1).

Histopathology. Necropsies were performed at 5, 12, and 18 days after inoculation with FLY or due to the different route of administration of pyran, pyran was inoculated i.v. and FLY was inoculated i.p. The disease process was still enhanced (Table 2; Fig. 1). Splenic foci were correspondingly increased whenever enhanced splenomegaly occurred. A decrease in survival time also occurred in mice in which FLY-induced splenomegaly was enhanced. A MST of 45 ± 2.9 days compared to a MST of 53 ± 2.0 days occurred in mice infected with FLY. In mice given pyran (12.5 mg/kg i.p.) against FLY i.p. infection, survival was prolonged (MST, 66 ± 2.0 days) (Chart 1).

Histopathology. Spleens were studied microscopically to determine whether i.v. administration of pyran increased the number of target cells for FLY. Mice were treated either i.p. or i.v. with pyran (12.5 mg/kg; Days —5 through —1) and were given injections i.p. of FLY or 0.15 M NaCl solution on Day 0.

The spleens of control animals that were given injections of 0.15 M NaCl solution only were morphologically similar at 5, 12, and 18 days (Fig. 2). The white pulp nodules comprised approximately 40 to 50% of the splenic substance seen in any given tissue section. Most nodules contained active germinal centers. The nodules were encircled by a clearly definable marginal zone that was 100 to 150 μm thick. The marginal zone contained cells with nuclei approximately twice the size of those of a small lymphocyte and...
Table 1
Reduction of splenomegaly caused by FLV in mice treated with i.p. pyran
Mice were inoculated i.p. with 1.2 x 10^6 focus-forming units of FLV. Ten days later spleens were removed from 10 mice/group, and spleen weights were determined as percentage of body weight ± S.E. Spleen weights of mice treated only with the drug ranged from 0.86 to 1.46 % body weight.

<table>
<thead>
<tr>
<th>Drug administration</th>
<th>Spleen wt (% body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus inoculation</td>
<td>Dose</td>
</tr>
<tr>
<td>0.15 M NaCl solution</td>
<td>None</td>
</tr>
<tr>
<td>FLV</td>
<td>None</td>
</tr>
<tr>
<td>FLV</td>
<td>125 mg/kg</td>
</tr>
<tr>
<td>FLV</td>
<td>25 mg/kg</td>
</tr>
<tr>
<td>FLV</td>
<td>25 mg/kg</td>
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Experiment 2
<table>
<thead>
<tr>
<th>Virus inoculation</th>
<th>Dose</th>
<th>Days</th>
<th>Foci*</th>
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</thead>
<tbody>
<tr>
<td>0.15 M NaCl solution</td>
<td>None</td>
<td>ND</td>
<td>0.45 ± 0.02</td>
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<tr>
<td>FLV</td>
<td>None</td>
<td>ND</td>
<td>3.70 ± 0.57</td>
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<td>FLV</td>
<td>25 mg/kg</td>
<td>-5, -4, -3, -2, -1</td>
<td>1.60 ± 0.50 (S)</td>
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<tr>
<td>FLV</td>
<td>25 mg/kg</td>
<td>-12, -11, -10, -9, -8</td>
<td>2.20 ± 0.53 (S)</td>
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<tr>
<td>FLV</td>
<td>25 mg/kg</td>
<td>-19, -18, -17, -16, -15</td>
<td>2.40 ± 0.58 (S)</td>
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<tr>
<td>FLV</td>
<td>25 mg/kg</td>
<td>-32, -31, -30, -29, -28</td>
<td>2.70 ± 1.04</td>
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</table>

* Foci on one-half of a splenic surface were counted.
* TNTC, foci too numerous to count (>100); S, p < 0.05; ND, not done.

Table 2
Enhancement or reduction of FLV splenomegaly depending on the route of pyran prophylaxis
Mice were treated with pyran (12.5 mg/kg) on Days -5 through -1 by the indicated route and were inoculated with 1.2 x 10^6 focus-forming units of FLV on Day 0. Ten days later spleens were removed from 10 mice/group, and spleen weights were determined as percentage of body weight ± S.E. Spleen weights of the mice treated only with the drug were 0.83 ± 0.05 % body weight for i.p. pyran and 1.73 ± 0.09 % body weight for i.v. pyran.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Route of drug administration</th>
<th>Spleen wt (% body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>i.p. inoculation</td>
<td></td>
</tr>
<tr>
<td>0.15 M NaCl solution</td>
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<td>0.58 ± 0.04</td>
</tr>
<tr>
<td>FLV</td>
<td>None</td>
<td>4.06 ± 0.53</td>
</tr>
<tr>
<td>FLV</td>
<td>i.p.</td>
<td>1.13 ± 0.03 (S)*</td>
</tr>
<tr>
<td>FLV</td>
<td>i.v.</td>
<td>9.96 ± 1.13 (S)</td>
</tr>
<tr>
<td></td>
<td>i.v. inoculation</td>
<td></td>
</tr>
<tr>
<td>FLV</td>
<td>None</td>
<td>7.19 ± 0.32</td>
</tr>
<tr>
<td>FLV</td>
<td>i.p.</td>
<td>7.45 ± 0.06</td>
</tr>
<tr>
<td>FLV</td>
<td>i.v.</td>
<td>12.82 ± 0.52 (S)</td>
</tr>
</tbody>
</table>

* S, p < 0.05.

with heterochromatin dispersed primarily as a thin layer at the periphery of the nucleus (primitive and stromal reticular cells). Some lymphocytes and erythrocytes were also present in this zone. The red pulp contained similar reticular cells, macrophages, and small groups of cells (i.e., clusters of 15 to 30 cells in the plane of section) involved in erythropoiesis (normoblasts and basophilic erythroblasts).

Five days after the i.p. injection of FLV, splenic morphology was much like that of the 0.15 M NaCl solution control, except for a slight increase in the number and size of erythropoietic centers. However, on Day 12 the red pulp consisted of a confluent mass of cells, morphologically similar to normoblasts and basophilic erythroblasts. By Day 18 the white pulp nodules were gradually forced farther apart and became hypocellular with germinal centers not being definable. The marginal zone became compressed nearly out of existence (Fig. 3).

The changes in the splenic morphology in normal mice after i.p. injection of pyran involved widening of the marginal zone surrounding white pulp nodules and an increase in the number and size of the regions of erythropoiesis (Fig. 4). These changes were slight at Day 5 and were extensive at Day 12 and 18. At the latter intervals, the
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The spleens of mice treated with i.v. pyran and infected with FLV showed a different pattern from the previous group. The initial changes in splenic morphology were the same as with i.v. pyran alone. At Day 5, the red pulp was characterized by vascular congestion and an apparent enhancement of erythropoiesis. The red pulp subsequently became a confluent mass of cells morphologically similar to normoblasts and erythroblasts. By Day 18, many of the white pulp nodules had been infiltrated with these cells and the marginal zone had become very narrow with its cells presenting the appearance of a stretched out fibrous capsule (Fig. 7); this was very similar to results with FLV treatment alone (Fig. 3).

DISCUSSION

Treatment of mice i.p. with pyran significantly protected them against i.p. challenge against FLV, as evidenced by reduced splenomegaly and splenic foci and prolonged survival time. These results are similar to those reported for other immunoadjuvants (4, 9, 17). The protection produced by this prophylactic regimen was also observed if FLV challenge was delayed for almost 3 weeks. The protection by this prophylactic treatment was maintained in some mice for as long as 100 days after virus challenge.

However, these results indicate that pyran can either protect or enhance leukemogenesis, by merely changing the route of drug administration. Treatment of mice i.v. with pyran followed by i.p. challenge with FLV significantly enhanced splenomegaly, increased splenic foci, and decreased survival time. Thus, by changing the route of pyran inoculation, a pronounced antileukemogenic effect was reversed to a potentiation of FLV disease.

Histological examination revealed a widening of the marginal zone after i.p. treatment with pyran. This is taken to represent a stimulation of the reticular cell pool (16) and is probably a morphological reflection of enhanced reticuloendothelial function commonly observed with pyran (15). In contrast to these results, after i.v. injection of pyran there was an increase in the relative amount of red pulp present at Day 5, earlier than the morphologically observable response after i.p. pyran. Since this change resulted from an increased number of erythroblasts, it seems evident that the enlargement of the pool of target cells (i.e., erythroblasts) occurs early after i.v. treatment with pyran. In addition, an enlargement of the marginal zone was not affected with the i.v. pyran regimen.

With the protective i.p. regimen of pyran followed by FLV, spleens appeared to be morphologically the same as with i.p. pyran alone. The marginal zone was enlarged, giving evidence of the reticuloendothelial stimulation. In contrast, with the i.v. regimen of pyran followed by FLV, the early response appeared primarily to involve an increase in the erythroblasts that are the target cells of the virus.

Therefore, histopathological examination of the spleens indicated that the protective i.p. pyran regimen may involve phagocytic cells. We have previously shown (11) that treatment of mice with pyran i.p. can activate macrophages to kill tumor cells nonspecifically and that macrophages are intimately associated with certain solid tumors in vivo after treatment of mice with pyran (M. G. Snodgrass, A. M. Kaplan, and P. S. Morahan, unpublished observations). In addition, Levy and Wheelock (13) have demonstrated that treatment of mice with silica, which caused a depression of macrophage function, interfered with the leukemia-suppressive activities of statolon, an immunopotentiator. These studies suggested that restoration of macrophage function may account for some part of the antileukemogenic effect of statolon, although a role for cytotoxic anti-FLV antibody was also indicated.

The i.v. regimen of pyran may increase the target cell pool for FLV, as evidenced by the early increase of the centers of erythropoiesis in the red pulp. In Rauscher leukemia virus-infected animals, these are equated to the "tumor cells" (18). The action of pyran in this regard may be similar to leukemogenic augmentation that has been noted by biological immunopotentiators (Bacillus Calmette-Guérin, complete Freund's adjuvant), by other synthetic
immunopotentiators (poly I:C, double-stranded RNA), by lactic dehydrogenase virus, and by leukogenenol (1, 7, 8, 12, 17, 19, 21). However, a common enhancing action has not been definitively established, and modulating effects on the immune system cannot be ruled out as a contributing factor.

REFERENCES

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