The Effect of Polyinosinic-Polyribocytidylic Acid on the Immune Response of Mice to Antigenically Distinct Tumors

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SUMMARY

Polyinosinic-polyribocytidylic acid temporarily inhibits the growth rate of visible, transplanted tumors of both chemical (methylcholanthrene-induced fibrosarcomas) and viral (murine polyoma) origin with nearly equal facility. This effect is dose related and is associated with lymphopenia and death at high doses or following prolonged administration. Polyinosinic-polyribocytidylic acid produces a variable effect on humoral antibody production in normal mice and can suppress cellular immunity in normal hosts, as evidenced by prolongation of skin allograft survivals in pretreated recipients. Prior host immunosuppression with either sublethal X-irradiation or antilymphocyte serum does not eliminate the inhibition of immunity in normal hosts, as evidenced by prolongation of skin allograft survivals in pretreated recipients. Prior host immunosuppression with either sublethal X-irradiation or antilymphocyte serum does not eliminate the inhibition of tumors provoked by polyinosinic-polyribocytidylic acid administration, thus suggesting that some mechanism other than stimulation of the immune response of the host is responsible for the effects of this drug on malignant cells.

INTRODUCTION

Levy et al. (11) have shown that double-stranded polyribonucleotides such as poly IC3 can inhibit the growth rates of certain experimental tumors. Bart et al. (2) have confirmed this observation, and Gelboin and Levy (8) have demonstrated that poly IC can depress oncogenesis as well. Included in a number of these reports is the suggestion that the antitumor effect seen might be related in some way to the well-known function of these drugs as antiviral agents, perhaps through their action as potent stimulators of interferon production. In addition, Turner et al. (15) and Cantor et al. (5) have reported that poly IC can stimulate both cellular and humoral immunity, thus suggesting yet another explanation for the tumor growth delay seen following its use, i.e., some form of nonspecific stimulation of the immune response of the host to cancer.

On the other hand, poly IC-induced inhibition does not appear to be limited to viral neoplasms, and certain highly immunogenic tumors of viral origin such as the SV 40 hamster tumor are apparently unaffected by poly IC (9). Furthermore, poly IC has been shown to be intrinsically toxic, leading to thymic atrophy, splenic hypoplasia, and lymphopenia following prolonged use (10). All of these observations would tend to cast suspicion on the idea that poly IC inhibits tumor growth by stimulating a more effective immune response.

This report provides further evidence that poly IC does not uniformly stimulate immune function but rather may be immunosuppressive when administered at a dosage level that ordinarily inhibits tumor growth. Furthermore, data are presented that indicate that poly IC inhibition of tumor growth is not dependent on the immunological integrity of the host.

MATERIALS AND METHODS

Tumors. Two isologous tumor-host systems were used: (a) methylcholanthrene-induced fibrosarcoma, maintained in the strain of origin, C57BL/6J mice, and (b) a polyoma that was obtained from Dr. Lloyd W. Law of the National Cancer Institute and maintained in C3H/HeJ mice. All mice used in these experiments were obtained from the Jackson Memorial Laboratories, Bar Harbor, Maine.

Poly IC. NaCl suspensions of poly IC were obtained from Dr. Hilton B. Levy of the National Cancer Institute, as well as from Microbiological Associates, Bethesda, Md. Poly IC was administered by i.p. doses ranging from 10 to 400 μg/day. In initial studies, administration of higher daily doses resulted in the rapid demise of large numbers of mice. Drug administration every 2nd day in lower doses produced less mortality and achieved readily measurable inhibition of tumor growth.

Measurement of Tumor Growth. Single-cell suspensions of tumor cells were prepared by enzyme digestion of minced tissue as described by Burk and Madden (4). Mice were challenged with 103 tumor cells in a hind leg, and after the appearance of visible and palpable tumors (approximately 3 x 3 mm), groups of 10 to 12 animals were given poly IC. Tumor-bearing controls received only 0.85% NaCl solution. Growth was followed by measurement of the tumor bidirectionally with a vernier caliper and by calculation of volumes according to the method of Attia and Weiss (1). Growth curves were plotted, and the significance of observed differences was tested by use of the Wilcoxon-Mann-Whitney rank order test (13). Significance was assigned to observations differing at p < 0.05 of random (chance) variance.

Assay of Humoral Immunity. Antibody response to SRBC was evaluated by use of a modification of the Jerne hemolytic plaque assay technique (14). Mice were splenectomized on the 4th day following i.v. injection of 0.25 ml of 25% packed SRBC (Colorado Serum Co., Denver, Colo.). Four mice were

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2 Present address: Department of Plastic Surgery, University of Virginia Medical Center, Charlottesville, Va. 22901.
3 The abbreviations used are: poly IC, polyinosinic-polyribocytidylic acid; SRBC, sheep red blood cells; ALS, antilymphocyte serum.
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used for each experimental variable, and plaque-forming cells were counted in triplicate. The data were compared by use of Student's t test.

**Assay of Cellular Immunity.** The effect of poly IC on cellular immune function was evaluated by measurement of its influence on the survival times of skin allografts exchanged across an H-2 histocompatibility barrier. Groups of 10 to 12 C3H/HeJ mice received skin grafts from C57BL/6J mice according to the technique described by Billingham and Silvers (3) and were immobilized for 1 week in plaster. Graft viability was then evaluated daily. The day of rejection was considered to be that point at which 10% or less of the graft was viable. Median survival times were plotted on log-probability paper according to the method of Litchfield (12). Significance of observed differences was tested by use of the Wilcoxon-Mann-Whitney rank order test (12).

**Immunosuppression.** Mice were immunosuppressed in 2 ways: (a) sublethal irradiation, i.e., 375 R delivered from a 250-kV, 50-ma orthovoltage unit without filtration (this represents 10% lethal dose that virtually eliminates antibody production as determined by hemolytic plaque assay and markedly diminishes circulating leukocytes); and (b) ALS, harvested from rabbits following sensitization with mouse thymocytes and Freund’s adjuvant according to a method previously described (6). Five injections of 0.1 ml of ALS were given s.c. on alternate days prior to tumor injection, a dosage that was known to be sufficient to prolong survival of skin allografts 18 to 26 days when control mice rejected grafts in 10.2 days.

**RESULTS**

**Effect of Poly IC on Tumor Growth.** Administration of poly IC i.p. resulted in a significant, although temporary, delay in the growth rates of experimental tumors tested. (Chart 1). Most of the tumors went on to kill their hosts, although about 4% of mice bearing polyomas showed total tumor regression following poly IC treatment. This effect was found to be dose related within the range of 10 to 400 µg/day (Chart 2). The use of this agent was not without side effects, however. Those mice receiving 400 µg/day died promptly after the initiation of treatment (within 3 to 5 days), although their tumors were

**Chart 1.** Effect of poly IC on tumor growth. Administration of poly IC i.v. resulted in a significant delay in the growth rates of both a methylcholanthrene (MCA) fibrosarcoma and a murine polyoma.

<table>
<thead>
<tr>
<th>Duration of poly IC pretreatment</th>
<th>Mean plaque-forming cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 µg/day</td>
<td>Mean plaque-forming cells</td>
</tr>
<tr>
<td>SRBC</td>
<td>Stimulative &lt;0.001</td>
</tr>
<tr>
<td>SRBC + poly IC</td>
<td>Stimulative &lt;0.001</td>
</tr>
<tr>
<td>Response</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td></td>
</tr>
<tr>
<td>t test</td>
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<td>1</td>
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<td>7</td>
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<td>15</td>
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</tr>
<tr>
<td>21</td>
<td>695</td>
</tr>
<tr>
<td>28</td>
<td>230</td>
</tr>
</tbody>
</table>

*a Poly IC given before SRBC administration plaquing performed 4 days following SRBC administration.

*b NS, not significant.
Effect of Poly IC on Immune Response of Mice

Table 2

Effect of poly IC on skin allograft survival (C57 → C3H)^

Prolongation of skin allograft survival was observed in mice receiving poly IC (100 μg, alternate days i.p.) prior to grafting and continuously to the day of rejection.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>No. of mice</th>
<th>Graft survival</th>
<th>Significance of prolongation</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>8</td>
<td>8–10</td>
<td>9.2</td>
</tr>
<tr>
<td>ALS, 0.1 ml s.c., Day -1</td>
<td>8</td>
<td>13–18</td>
<td>14.2</td>
</tr>
<tr>
<td>Poly IC, 100 μg alternate days i.p., Day -1</td>
<td>10</td>
<td>9–11</td>
<td>9.2</td>
</tr>
<tr>
<td>Poly IC (continuous) from Day -7 to day of rejection</td>
<td>13</td>
<td>11–16</td>
<td>13.3</td>
</tr>
</tbody>
</table>

^

a Grafts placed Day 0.

b NS, not significant.

regressing. Lymphopenia was another commonly observed result of poly IC administration (Chart 3).

Effect of Poly IC on Humoral and Cellular Immunity of Normal Mice. Groups of 10 to 12 normal mice were pretreated with poly IC (100 μg i.p. on alternate days) for periods ranging from 1 to 28 days prior to hemolytic plaque assays. The results (Table 1) showed that antibody responsiveness 4 days following SRBC administration was quite variable. Those mice receiving poly IC for 1 to 7 days demonstrated plaque-forming cell counts significantly less than control groups receiving only 0.85% NaCl solution. After longer periods of poly IC administration, i.e., 7 to 28 days, similar to that required to
produce tumor inhibition, the response was stimulated in some groups and significantly inhibited in others without any apparent predictable pattern.

Poly IC produced a depressive effect on cellular immunity (Table 2). Skin allografts exchanged between H-2-incompatible strains (C57BL/6–C3HHeJ) remained viable for significantly longer periods of time than controls when poly IC was given prior to grafting and continued (100 μg i.p. on alternate days) until the day of rejection. Shorter periods of poly IC treatment did not result in prolonged allograft survival. The degree of immunosuppression seen was approximately equivalent to that seen following the administration of 0.1 ml of ALS.

**Effect of Host Immunosuppression on Poly IC Inhibition of Tumor Growth.** For determination of the role of the immune system in the observed effects of poly IC on tumor growth, several groups of 10 to 12 mice each were immunosuppressed with either sublethal irradiation or ALS and then challenged with 10³ tumor cells. Following the appearance of palpable tumors, poly IC treatment was begun (100 μg i.p. 3 times weekly). Controls received 0.85% NaCl solution with and without immunosuppression. The resultant growth curves are shown in Charts 4 to 7. Both the chemically induced fibrosarcoma and the polyoma were inhibited despite host immunosuppression. All groups receiving ALS exhibited faster tumor growth than non-ALS controls (Charts 6 and 7), a finding in agreement with those previously reported [7]. However, poly IC recipients in each experiment continued to show a proportionately slower growth rate than NaCl solution controls regardless of whether or not they received prior irradiation or ALS administration. Furthermore, in order to be certain that poly IC was not simply reversing the immunosuppressive effects of ALS, skin allograft survivals were compared between mice receiving both ALS and poly IC and those receiving ALS alone and were found to be nearly identical (27.2 days versus 29.4 days).

**DISCUSSION**

The mechanism of action of poly IC inhibition of experimental tumor growth is not clear. Although synthetic polyanucleotides have generally been considered to be stimulants of immune function, these studies provide evidence that poly IC can also depress both humoral and cellular immunity, particularly after the prolonged periods of administration necessary to achieve tumor inhibition. In addition, the present studies have shown that poly IC effectively inhibits tumor growth in immunosuppressed mice. Thus it appears that a competent immune system is not necessary for poly IC to manifest its antitumor effects.

The suggestion that poly IC inhibits tumor growth by its antiviral activity appears unlikely, since chemically induced tumors are inhibited as well as some viral tumors. Of course, it might be contended that the development of chemically induced neoplasms may in fact be virus mediated, but there is very little evidence to support this idea. Furthermore, the resistance of SV 40-induced tumors to the inhibitory influence of poly IC (9) also casts doubt on the involvement of an antiviral mechanism in the action of poly IC.

Other possible antitumor mechanisms include a direct cytotoxic action on tumor cells, a suggestion that is partially supported by the marked lymphopenia that attends the use of poly IC, as well as the frequency with which lethal complications occur following administration of doses in excess of 100 μg/day in the mouse. At present, however, there are no adequate data regarding the effects of synthetic polyanucleotides on cellular replication in vitro. Antitumor defenses such as enhanced chalone production or synthesis of other poorly defined natural growth-inhibitory substances may be stimulated by poly IC, but for the present these possibilities are only conjectural. Further study of the mode of action of poly IC is clearly needed and may open new doors to understanding the normal host mechanisms for control of neoplastic development and growth.

**REFERENCES**

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