Immunotherapeutic Potential in Murine Tumor Models of Polyinosinic-Polyctydilic Acid and Poly-L-lysine Solubilized by Carboxymethylcellulose

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ABSTRACT

The systemic administration of multiple, nontoxic doses of polyinosinic-polycytidylic acid and poly-L-lysine solubilized by carboxymethylcellulose [poly(I,C)-LC] eradicated established experimental and spontaneous pulmonary metastases. Optimal immunotherapy was schedule dependent, requiring three to five injections of poly(I,C)-LC per week for a minimum of 4 weeks; in addition, therapeutic efficiency was partially dosage independent. Immunotherapy by poly(I,C)-LC was found to be limited by tumor burden, although when combined with chemotherapy as a debulking regimen it resulted in increased survival with protocols in which poly(I,C)-LC alone was insufficient. These data suggest that the systemic administration of poly(I,C)-LC may provide a successful adjuvant therapeutic modality against cancer metastasis.

INTRODUCTION

The complex of poly(I,C) and poly-L-lysine which is solubilized by the addition of carboxymethylcellulose [poly(I,C)-LC] is an excellent interferon inducer in nonhuman primates and in humans (3-5, 16, 17, 19, 22-25, 35). In primates, the effectiveness of poly(I,C)-LC compared with poly(I,C) appears to be due to its 4- to 10-fold increased resistance to hydrolysis (1, 17, 23, 24), resulting in increased stability.

The initial pharmacological approach to the study of BRM is to examine their effects in vitro on the effector cell populations of normal animals (8, 26, 33). These studies provide a preliminary assessment of the immunomodulatory activity at the level of individual immune functions. However, these in vitro observations are not necessarily representative of in vivo events induced by a BRM; thus, the next step in the pharmacological approach is to determine the immunomodulatory properties in vivo. The comparison of in vitro and in vivo immunomodulation provides data on the BRM-host interactions, including degradation, inhibition, and any requirement for metabolism or cellular corporation to express specific immunopharmacological properties as well as dosage information and host toxicity.

Upon conclusion of these preliminary investigations, the preclinical screening of a BRM should include an assessment of its immunotherapeutic properties (8, 33). The immunotherapy models need to incorporate an assessment of the role of tumor cell burden at the initiation of treatment and the effect of tumor site on tumor therapy (33). Furthermore, the dosage, schedule, and route of administration can greatly affect the response of the host to BRMs in a quantitative and qualitative manner. Therefore, in addition to determining the therapeutic efficacy of the immunomodulator, it is necessary to determine the optimal therapeutic doses, which may differ from an optimal immunomodulatory dose as determined in normal animals or the maximum tolerated dose. In addition, we examined the optimal treatment parameters the optimal scheduling and duration of poly(I,C)-LC administration.

As previously reviewed (31, 33), we believe that the treatment of systemic disease provides the best model of therapeutic efficacy. Using poly(I,C)-LC, we undertook studies to determine its immunomodulatory properties (29) and to elucidate the optimal protocol for therapeutic efficacy, which should provide guidance for the design of future clinical trials.

MATERIALS AND METHODS

Animals. Specific-pathogen-free male C57BL/6N mice (H-2b) and C3H/HeN (MTV-) mice (H-2b), 4 weeks of age, were obtained from the Animal Production Area of the National Cancer Institute-Frederick Cancer Research Facility. These mice were used as age-matched cohorts from 6 to 8 weeks of age.

Tumors. These studies used the metastatic variant obtained from the radiation-induced fibrosarcoma UV-2237 (15), syngeneic to the H-2b C3H mouse, which is referred to as UV-2237 Met-Mix (14). We also used the metastatic melanoma variant, B16 BL6 (12), which was selected in vitro from the B16 melanoma, a spontaneous tumor from a C57BL/6N (H-2b) mouse. The fibrosarcoma and melanoma were maintained as monolayers in Eagle’s minimal essential medium supplemented with 5% fetal bovine serum, sodium pyruvate, nonessential amino acids, glutamine, and a 2-fold vitamin solution. All cell lines were free of Mycoplasma and pathogenic murine viruses (30).

Agents. Poly(I,C)-LC was generously provided by Dr. Hilton Levy, National Institute of Allergy and Infectious Disease, Frederick, MD. Thymosin Fraction 5 was provided by Hoffmann-La Roche Inc., Nutley, N.J. MTP-PE was provided by Ciba Geigy, Summit, N.J. Poly(I,C) was obtained from Sigma Chemical Co., St. Louis, MO. Phosphatidylserine and phosphatidylethanolamine were obtained from Avanti Polar Lipids, Birmingham, AL.

Immunization. The immunization protocol involved a suboptimal vaccine of irradiated, collagenase-dissociated UV-2237 tumor cells (1 x 10⁶), with or without an adjuvant, into syngeneic mice, at an i.d. site. Subop-
tinal vaccines are used to allow the demonstration of an adjuvant-like activity (30). Vaccines consisted of tumor cells admixed with HBSS or tumor cells admixed with one of several different doses of poly(I,C)-LC in a volume of 0.05 ml/site. Control vaccines consisted of HBSS alone or poly(I,C)-LC alone. The mice were challenged 10 days following inoculation with an intrafootpad injection of 1 x 10^6 UV-2237 Met-Mix tumor cells obtained by the collagenase dissociation of a s.c. tumor (30).

**Therapy of Established Metastases.** Syngenic C57BL/6N mice or C3H/HeN (MTV-) mice at 8 weeks of age were given i.v. injections of single cell suspension of 25,000 in vitro propagated B16-BL6 melanoma cells (C57BL/6 mice) or 50,000 in vitro propagated UV-2237 Met-Mix fibrosarcoma cells (C3H/HeN) in CMF-HBSS (0.2 ml). The schedule of therapeutic injections varied for each experiment and are described within the text. Therapy was continued for 1 to 4 weeks; the mice in the experimental groups that were alive 3 weeks after the last control animal had died were killed, and necropsies were performed. Therapeutic efficacy was determined based on the survival of the mice, percentage of tumor-free animals, or the extent of pulmonary metastases.

The therapeutic efficacy of poly(I,C)-LC on spontaneous metastases from B16-BL6 melanoma was also evaluated. B16-BL6 melanoma cells (5 x 10^5) in 0.05 ml of CMF-HBSS were inoculated into the posterior footpads of 8-week-old syngeneic mice. When the primary tumor reached a diameter of 0.8 to 1 cm, the tumor-bearing leg was resected at midfemur to include the popliteal lymph node. Seventy-two hr later, therapy was initiated, using a protocol of twice-weekly injections for a total of 4 weeks. Necropsies were performed 4 weeks following the last injection, the lungs were fixed in Bouin's fixative to contrast the tumor foci to host parenchyma, and the extent of spontaneous pulmonary metastases was determined by counting the metastases under a dissecting microscope.

**Statistical Analyses.** The difference between the extent of metastasis (experimental or spontaneous) between control, CMF-HBSS-treated animals, and experimental groups was determined using the nonparametric Mann-Whitney U test. Ten animals were included in each group. The tumor volumes were examined weekly by measuring the tumor diameter in 2 dimensions. The tumor volume was determined using the formula for a prolated sphere:

\[ V = 0.5 \times a \times b^2 \]

where a is the large diameter, and b is the small diameter. Survival data were analyzed using the Kruskal Wallis analysis as adapted for censored observation by Gehan and Breslow (34) and the Cox proportional hazards model.

**RESULTS**

**Nonspecific Immunoprophylaxis of Poly(I,C)-LC on Experimental Metastases.** Three-week-old C3H mice received a single injection of poly(I,C)-LC and 24 hr later were challenged by the i.v. injection of a single cell suspension of 50,000 UV-2237 Met-Mix tumor cells. Necropsies were performed 21 days later and the extent of pulmonary colonization was determined. In this assay of immunoprophylaxis, poly(I,C)-LC significantly reduced the extent of experimental metastases (Table 1) as well as the number of mice which developed lung nodules. The pretreatment of mice with equal doses (total weight basis) of poly IC or poly(I,C)-LC induced similar resistance to the development of experimental metastases. The prophylaxis induced was dosage dependent; the highest doses of poly(I,C)-LC, 2.5 or 5 mg/kg, were most effective in preventing the development of lung nodules. The lowest doses of poly(I,C)-LC (0.05 mg/kg) were less effective, but they still significantly reduced the median number of lung colonies produced by the i.v. injection of tumor cells.

**Special Immunoprophylaxis.** The immunoadjuvant activity of poly(I,C)-LC was examined as an admixture with a tumorspecific vaccine (30) and the efficiency assessed by the ability to prevent the outgrowth of a tumor cell challenge. Normal syngeneic mice were immunized by the intradermal injection of lethally irradiated tumor cells obtained from collagenase-dissociated tumors. Mice which received the suboptimal tumor vaccine, consisting of irradiated tumor cells in HBSS, developed tumor numbers and sizes similar to those found in control mice which received HBSS alone (Table 2). In contrast, mice immunized with tumor cells admixed with poly(I,C)-LC or the positive control thymosin Fraction 5 had a higher degree of protection against tumor challenge. This adjuvant-like effect of poly(I,C)-LC was dose dependent, requiring 0.25 or 2.5 mg/kg of poly(I,C)-LC.

**Therapy of Experimental Metastatic Foci Using Poly(I,C)-LC.** The therapeutic potential of poly(I,C)-LC was investigated.
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using both experimental and spontaneous metastases models. In the experimental metastasis model, therapy was initiated 3 days following the i.v. injection of B16-BL6 tumor cells (Chart 1). At this time, multiple microfoci of pulmonary tumor nodules are histologically evident. Poly(I,C)-LC significantly reduced the number of experimental metastases in a dose-dependent manner and significantly prolonged the survival of mice bearing experimental metastases. Poly(I,C)-LC prolonged survival at 0.05 and 0.5 mg/kg (Cox's test 0.014 and 0.035, respectively) and generalized Kruskel Wallis analysis (0.014 and 0.047, respectively), with 0.05 mg/kg poly(I,C)-LC having significantly better therapeutic benefit in this experiment than 0.5 mg/kg poly(I,C)-LC. Furthermore, treatment of animals with preexistent experimental metastases with a 0.05-mg/kg dose of poly(I,C)-LC "cured" 30% of the animals. In a subsequent experiment (Table 3), syngeneic mice bearing UV-2237 Met-Mix pulmonary nodules were treated with poly(I,C)-LC or MTP-PE starting either 2 or 8 days following tumor challenge. In this study, poly(I,C)-LC at 1, 0.5, and 0.25 mg/kg was equally effective in reducing the number of pulmonary metastases in the animals in which treatment was started 2 days following tumor challenge. In contrast, when therapy was delayed until 8 days following tumor challenge, only poly(I,C)-LC at 1 mg/kg and liposomes incorporating MTP-PE were effective in significantly reducing the number of pulmonary metastases. The lower doses of poly(I,C)-LC were not efficacious against this increased tumor burden. Thus, it appears that although lower doses of poly(I,C)-LC may be effective against minimal tumor burden, higher doses of poly(I,C)-LC may be required in animals bearing extensive tumor burden.

Therapy of Spontaneous Metastases. The therapy of spontaneous metastases by the administration of poly(I,C)-LC twice weekly for 4 weeks also resulted in an immunotherapeutic response (Table 4). Mice were entered onto this therapeutic protocol 3 days following the resection of the primary B16-BL6 footpad tumor. Mice that received excipient (Hanks' balanced salt solution) injections developed a median of 10 spontaneous metastases, with 70% of the animals remaining tumor-free at necropsy. Mice treated with poly(I,C)-LC at 0.25 mg/kg had a median of 3 pulmonary metastases, with 50% of the animals remaining tumor-free after the last injection (n = 10 mice; occasionally an animal was censored due to nonrelevant events).

Table 3

<table>
<thead>
<tr>
<th>BRM</th>
<th>Initiation of therapy (day)</th>
<th>Median no. of lung colonies</th>
<th>% of tumor-free mice</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBSS</td>
<td>+2</td>
<td>17 (4–38)</td>
<td>0</td>
<td>=0.005</td>
</tr>
<tr>
<td>MTP-PE</td>
<td>+2</td>
<td>3 (0–11)</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Poly(I,C)-LC</td>
<td>1 mg/kg</td>
<td>+2</td>
<td>1 (0–4)</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>0.5 mg/kg</td>
<td>+2</td>
<td>1 (0–5)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>0.25 mg/kg</td>
<td>+2</td>
<td>1 (0–13)</td>
<td>40</td>
</tr>
<tr>
<td>HBSS</td>
<td>+8</td>
<td>19 (11–42)</td>
<td>40</td>
<td>0.01</td>
</tr>
<tr>
<td>MTP-PE</td>
<td>+8</td>
<td>8 (0–51)</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Poly(I,C)-LC</td>
<td>1 mg/kg</td>
<td>+8</td>
<td>7 (0–66)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>0.5 mg/kg</td>
<td>+8</td>
<td>22 (0–57)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>0.25 mg/kg</td>
<td>+8</td>
<td>27 (0–52)</td>
<td>20</td>
</tr>
</tbody>
</table>

* Probability of no difference in the number of nodules compared to the mice which received the HBSS control as determined using the Mann-Whitney U test.

** NS, not significant.

Table 4

Therapy of spontaneous B16-BL6 metastases by poly(I,C)-LC

B16-BL6 tumor cells were injected into posterior footpads of each mouse. When the tumors reached a 0.8-cm diameter, the tumor-bearing limb was resected at midfemur to include the popliteal lymph node. Therapy was initiated 3 days later with twice weekly i.v. injections for 4 weeks. Necropsies were performed 3 weeks after the last injection (n = 10 mice; occasionally an animal was censored due to nonrelevant events).

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose (mg/kg)</th>
<th>% of mice with metastases</th>
<th>Median no. of metastases</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBSS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(I,C)-LC</td>
<td>2.5</td>
<td>30</td>
<td>20 (0–20)</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>50</td>
<td>30 (0–20)</td>
<td></td>
</tr>
<tr>
<td>MTP-PE</td>
<td>2.5</td>
<td>50</td>
<td>30 (0–20)</td>
<td></td>
</tr>
<tr>
<td>Poly(I,C)-LC</td>
<td>1.25</td>
<td>45</td>
<td>30 (0–20)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>45</td>
<td>30 (0–20)</td>
<td></td>
</tr>
</tbody>
</table>

* Probability of no difference from the HBSS control as determined using the Mann-Whitney U test.

** Numbers in parentheses, range.

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poly(l,C)-LC has therapeutic efficacy, not only against the limited tumor burden seen with experimental metastases but also against well-established spontaneous metastases in a tumor-compromised host.

Scheduling of Immunotherapy with Poly(l,C)-LC. Optimal scheduling of poly(l,C)-LC immunotherapy was investigated using the experimental metastasis model where therapy was initiated 3 days following tumor challenge. Chart 2 represents the results of a study to determine the optimal number of injections per week of poly(l,C)-LC at 1.25 mg/kg administered 1, 2, or 3 times a week for 4 weeks. Significant prolongation of survival was observed when poly(l,C)-LC was given twice (p = 0.03) or 3 times (p = 0.001) per week. No significant prolongation of survival was observed in animals treated once per week compared to animals treated with 0.9% NaCl solution (saline). The 3-times-weekly administration of poly(l,C)-LC provided significantly better protection than did the twice-weekly administration (p = 0.005). Because necrosis developed at the sites where multiple i.v. injections were given, following the first 8 i.v. injections the remainder were given i.p. Similar schedule dependency was observed in studies with ascites MBL-2 tumor-bearing animals, with treatment 3 times weekly providing optimal therapeutic efficacy.

To determine whether the administration of poly(l,C)-LC for 4 weeks was required or if a shorter schedule would be sufficient, poly(l,C)-LC was delivered on a suboptimal, twice-weekly schedule for 1, 2, 3, or 4 weeks beginning 3 days following tumor challenge. As shown in Table 5, no therapeutic efficacy was observed when mice were treated with poly(l,C)-LC twice a week for only 1 or 2 weeks; however, at 0.5 mg/kg of poly(l,C)-LC, a significant decrease in the median number of metastases was observed when it was administered for 3 weeks (p = 0.027; Kruskal Wallis analysis). When therapy was continued for 4 weeks (both 0.5 and 2.5 mg/kg), it resulted in a significant prolongation of survival as determined by Cox’s analysis (p = 0.0013 and 0.0007, respectively).

Additional experiments were designed to determine the therapeutic efficacy of multimodality therapy using poly(l,C)-LC and the chemotherapeutic agent cyclophosphamide. In this series of experiments (Chart 3), the lymphoid tumor line, MBL-2 (28), was injected i.p. (2 x 10⁶ cells/ml) into syngeneic C57BL/6N mice. On Day 1, mice received cyclophosphamide (200 mg/kg), and various experimental groups received one injection of poly(l,C)-LC at 1 mg/kg on Day 7 or multiple injections on Days 7 and 14 or Days 7, 14, and 21. Treatment with cyclophosphamide alone significantly prolonged survival (p = 0.0002; Cox’s analysis) as did the treatment protocols using cyclophosphamide and poly(l,C)-LC. Treatment with both agents prolonged survival better than either agent alone, as determined using Cox’s analysis (Day 7, p = 0.021; Days 7 and 14, p = 0.015; Days 7, 14, and 21, p = 0.002). Experiments with cyclophosphamide (100 mg/kg) and 1, 2, or 3 injections of poly(l,C)-LC resulted in similar improvements in survival. In other studies, (results not shown) in which treatment with cyclophosphamide was delayed, multiple injections of poly(l,C)-LC on Days 11, 18, and 25 combined with cyclophosphamide (200 mg/kg) administered on Day 5 or multiple injections of poly(l,C)-LC on Days 7, 14, and 21 when cyclophosphamide (100 mg/kg) was given on Day 1 were required to provide a significant increase in the survival of the treated animals. Similar studies using experimental lung metastases of this fibrosarcoma UV-2237 mm and treatment with mitomycin C, with or without poly(l,C)-LC, also demonstrated improved survival (as well as reduced metastases) in animals receiving combined treatment compared to either agent alone.

**DISCUSSION**

Despite substantial successes with immunomodulators in animal models, clinical trials have generally been disappointing, with results that have been inferior to those obtained with a variety of animal tumor models (7, 8, 26, 31, 32). The positive results observed with experimental tumor systems appear to be due, in part, to the initiation of therapy in animals with minimal tumor burdens. The difficulty in translating these positive results into a clinical setting is predictable, since it is unusual for a patient to present with a circumscribed lesion containing only 10⁵ to 10⁶ tumor cells of 24-hr duration. Regardless of this disparity in tumor burden between animal models and the clinical situation, the immunotherapeutic “cure” of even minimal tumor burdens could lead to the development of a novel agent or approach capable of inhibiting larger tumor burdens. Since metastasis has frequently occurred in the cancer patient at the time of diagnosis and is the predisposing cause of death, the major problem in the treatment of the majority of neoplasms is the elimination or control of metastases (7). The identification and development of BRM for clinical oncology require the development of effective protocols using hosts with pre-existent, well-established metastases.

Clearly, the development of BRM requires new strategies. Rather than determine only the MTD working within the framework of escalating doses, it may prove advantageous to also determine the optimum immunomodulatory dose as well as
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Table 5

Therapy of experimental metastases with poly[(I,C)-LC]: length of therapy

Syngeneic mice (C57BL/6) were given 5 x 10^6 B16-BL6 tumor cells by i.v. injection, and immunotherapy was initiated 2 days later. Therapy consisted of the twice-weekly i.v. injection of each BRM for 1, 2, 3, or 4 consecutive weeks. Necropsies were performed on the mice, and the extent of experimental metastases was determined with the aid of a dissecting microscope. Surviving mice were necropsied on Day 72 and reported to be tumor free if no nodules were apparent by gross examination with a dissecting microscope (n = 10).

<table>
<thead>
<tr>
<th>BRM</th>
<th>Dose (mg/kg)</th>
<th>Schedule (times/wk)</th>
<th>Length (wk)</th>
<th>Median no. of lung colonies</th>
<th>% of tumor-free mice</th>
<th>Mean survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBSS</td>
<td>2.5</td>
<td>2</td>
<td>4</td>
<td>&gt;300 (24-&gt;300)</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>Poly[(I,C)-LC]</td>
<td>2.5</td>
<td>2</td>
<td>1</td>
<td>&gt;300 (24-&gt;300)</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>Poly[(I,C)-LC]</td>
<td>2.5</td>
<td>2</td>
<td>2</td>
<td>147 (3-&gt;300)</td>
<td>0</td>
<td>36</td>
</tr>
<tr>
<td>Poly[(I,C)-LC]</td>
<td>2.5</td>
<td>2</td>
<td>3</td>
<td>&gt;300 (0-&gt;300)</td>
<td>10</td>
<td>39*</td>
</tr>
<tr>
<td>Poly[(I,C)-LC]</td>
<td>2.5</td>
<td>2</td>
<td>4</td>
<td>35* (0-&gt;300)</td>
<td>20</td>
<td>40*</td>
</tr>
<tr>
<td>Poly[(I,C)-LC]</td>
<td>0.5</td>
<td>2</td>
<td>1</td>
<td>&gt;300 (24-&gt;300)</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>Poly[(I,C)-LC]</td>
<td>0.5</td>
<td>2</td>
<td>2</td>
<td>&gt;300 (19-&gt;300)</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>Poly[(I,C)-LC]</td>
<td>0.5</td>
<td>2</td>
<td>3</td>
<td>74* (12-&gt;300)</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>Poly[(I,C)-LC]</td>
<td>0.5</td>
<td>2</td>
<td>4</td>
<td>40* (0-&gt;300)</td>
<td>20</td>
<td>44*</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, range.

† Probability of no difference in the number of lung nodules compared to the mice which received the HBSS control as determined using the Mann-Whitney U test (p < 0.05).

‡ Probability of no difference in survival compared with the HBSS control by the Cox's analysis (p < 0.05).

determine the optimal schedule and duration for immunotherapy. In general, tumor treatment may take 2 forms during experimental studies. The agent can be administered prior to or simultaneously with tumor challenge (prophylaxis), a protocol with obvious disparities from the clinical situation, or therapy can be initiated following tumor challenge. These modes of therapy may be further delineated based on the injection schedule, nature of the targeted effector cells, and site of tumor challenge.

The immunophrophylaxis studies may be non-tumor-specific, which may be dependent on NK cells (10, 11, 32, 36), or specific vaccines using tumor cell vaccination of syngeneic mice followed by tumor challenge. In the nonspecific assay of immunophrophylaxis, poly[(I,C)-LC] administered 24 hr prior to tumor challenge significantly depressed the outgrowth of experimental metastases in a dose-dependent manner. The dose-dependent immunophrophylaxis paralleled the induction of splenic NK-cell activity reported previously (29). Whether this effect was due to immune enhancement, tumor inhibition by the interferon-induced, specific effect of poly[(I,C)-LC], tumor macromolecule synthesis (25), or induction of other lymphokines is difficult to say. Pol'y[(I,C)-LC] also had therapeutic benefit associated with a dose-dependent adjuvant-like activity. However, high doses of poly[(I,C)-LC] were less efficacious in this model than low doses, presumably due to the loss of metabolically viable cells in the vaccine, which prevented the chronic release of tumor antigens.

However, successful immunophrophylaxis in animal models is not predictive for clinical success. However, clinical efficiency may be associated with therapeutic studies against pre-existent systemic disease. This approach may be directed against experimental metastases or against spontaneous metastases following primary tumor resection. Poly[(I,C)-LC] was found to have therapeutic value as assessed in both of these models of metastasis [those of limited duration (experimental metastasis) or systemic disease in a tumor-conditioned animal (spontaneous metastasis)]. In contrast to the approaches traditionally used with cytotoxic drugs, immunotherapy with poly[(I,C)-LC] did not require near-toxic doses of poly[(I,C)-LC]. Indeed, lower doses on an optimal schedule (2 to 3 injections/week for at least 4 weeks) were equally effective statistically as higher doses on the same therapeutic schedule. Nonetheless, optimal dosages may need to be adjusted on an individual basis as determined by effector cell stimulation. Based on our present results, a therapeutic protocol with poly[(I,C)-LC] administered 3 times/week and continued for a minimum of 4 weeks provides near-optimal therapeutic benefit that is achieved at nontoxic levels of poly[(I,C)-LC]. In contrast to the maintenance of NK cell activity by the weekly injection of poly[(I,C)-LC] (29), no therapeutic efficacy was associated with the weekly injection of poly[(I,C)-LC]. This suggests that NK cells may not be the important effector cell in the immunotherapeutic treatment of metastatic disease by poly[(I,C)-LC].

Whether these recommendations will translate into a clinically effective protocol remains to be seen. Nonetheless, it is readily
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apparent that rather than approaching therapeutic protocols based on a MTD, it may be more advantageous to deliver an optimal modulatory dose. However, this may vary depending upon the patient’s tumor burden, and an optimal modulatory dose should be delivered on a schedule sufficient to ensure continuous immunostimulation. Furthermore, this schedule should be maintained for several months to achieve maximal therapeutic effect. At present, we believe that NK cell augmentation, monocyte activation, and T-cell parameters should be included in the monitoring studies. We suggest that therapeutic protocols should be developed that result in the continued monocyte stimulation and the restoration-stimulation of T-lymphocyte function.

Several clinical trials have been performed with poly(I,C)-LC using either a constant dose and different schedules (2, 13, 18, 20–23) or dose-escalating schedules (16–18, 20). These Phase I and II studies have included patients with neurological disorders (2, 6, 16, 17) and neoplastic disease (13, 18, 20–23). Doses of poly(I,C)-LC >12 mg/sq m were in general found to be intolerable, while doses between 1 and 4 mg/sq m had lesser toxicity. Most ongoing Phase I and II trials use doses from 1 to 8 mg/sq m, and levels of 1 mg/m may prove necessary for maintenance immunotherapy. The IFN levels observed in these trials peak 4 to 12 hr following poly(I,C)-LC administration and may last for up to 36 hr (13). Increased NK cell cytotoxicity has also been noted in several patients with high serum titers of IFN as well as in those with no significant serum IFN (13, 20–23). Ongoing trials by the Biological Response Modifiers Program of the National Cancer Institute are administering poly(I,C)-LC iv. or i.m. at 1 and 4 mg/sq m with the observation of variable immune modulation. Extensive studies are being undertaken to fully evaluate the effects of these 2 doses of poly(I,C)-LC on NK-cell cytotoxicity, monocyte cytostasis, T-cell subset quantitation, 2’-5’-A synthetase levels, and IFN induction.6 In this study as well as others (13, 20–23), there has been no direct correlation between NK activity, the dose of poly(I,C)-LC given, and serum IFN levels obtained. However, in the Biological Response Modifiers Program study, significantly increased monocyte cytostasis has been a consistent observation at both doses of poly(I,C)-LC administration. It appears, in general, that within most Phase I trials, the nature of immunomodulation associated with poly(I,C)-LC parallels that observed in the animal models. However, the brief period of immunotherapy (1 to 4 weeks in most trials) which is associated with either a single dose or escalating doses, all of which have been near the MTD, need not provide optimal therapeutic benefit.

Host defenses following BRM activation are capable of controlling only a small number of tumor cells (up to 5 x 10^7), while the treatment of a more extensive tumor burden (10^9 tumor cells) generally results in progressive disease (9, 27). Because the detection of neoplastic disease generally requires a discrete lesion 0.8 to 1.0 cm in diameter, which contains approximately 10^8 tumor cells, it is unlikely that tumors will be diagnosed at a sufficiently early stage to allow successful immunotherapy. One such approach is multimodal therapy incorporating both chemotherapeutic and immunotherapy. In such cases, immunotherapy may have a role as a therapeutic adjunct against clinically inapparent secondary disease or in conjunction with debulking mo-

dalities of treatment. This approach is demonstrated in Chart 3 where a cytotoxic agent (cyclophosphamide) provided a slight prolongation of survival but, in conjunction with a suboptimal immunotherapy regimen with poly(I,C)-LC, provided significant therapeutic efficacy. Thus, when cytostatics are used as a debulking protocol, even ineffective immunomodulatory protocols (when used alone) may greatly prolong or, indeed, "cure" tumor-bearing hosts.

REFERENCES


Immunotherapeutic Potential in Murine Tumor Models of Polyinosinic-Polycytidylic Acid and Poly-I-lysine Solubilized by Carboxymethylcellulose


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