Maleic Vinyl Ether Activation of Murine Macrophages against Lung-metastasizing Tumors

Scott E. Loveless and Albert E. Munson

ABSTRACT

A 15,500 molecular weight fraction of maleic vinyl ether (MVE-2) induced tumoristatic and tumoricidal activity in peritoneal macrophages of BALB/c and C57BL/6 mice following i.p. administration. Growth of B16 melanoma cells in vitro was inhibited up to 85% by MVE-2-activated, but not resident, peritoneal macrophages. In a tritiated thymidine release assay, B16 melanoma cells, and to a lesser extent Madison 109 lung carcinoma cells, were also sensitive to the cytolytic action of MVE-2-activated peritoneal macrophages.

Administration i.v. of MVE-2 resulted in tumoristatic and tumoricidal activity in alveolar macrophages against radiolabeled B16 and Madison 109 lung carcinoma target cells. MVE-2-activated alveolar macrophages significantly inhibited L5178Y lymphoma colony formation following a 48-hr macrophage-tumor cell cocultivation.

BALB/c mice bearing the lung-metastasizing Madison 109 lung carcinoma footpad tumor were given MVE-2 i.v., using the same dosing regimen that induced alveolar macrophages to be tumoricidal in vitro. Significant increases in life span were observed, suggesting that the antitumor activity of MVE-2 in this tumor system may be mediated by the activation of alveolar macrophages, with a resulting decrease in metastatic growth in the lung.

INTRODUCTION

The macrophage has been implicated as an important cell type in host defense against neoplastic disease (7, 13). Animals have been found to be more resistant to the growth of certain tumors following administration of macrophage activators, such as BCG (29), Corynebacterium parvum (24), and MVE (15), frequently referred to as pyran copolymer (6, 8, 17, 23). Administration of agents toxic to macrophages can enhance the interactions between alveolar macrophages and neoplastic cells (27). Since the lung is a frequent site of metastatic growth, the importance of investigating the tumoricidal activity of alveolar macrophages is clear. The present studies were undertaken to determine whether MVE-2, a low-molecular-weight (M.W. 15,500) fraction of MVE, could activate in vivo murine peritoneal and alveolar macrophages to kill tumor cells in vitro and, if so, whether a therapeutic protocol could be developed which would increase the life span of mice bearing the metastatic M109 lung carcinoma.

MATERIALS AND METHODS

Mice

Male BALB/c and C57BL/6 mice, 8 to 12 weeks old, were obtained from Simonsen Laboratories, Gilroy, Calif. The mice were housed 5/cage and were maintained on Purina laboratory chow (Ralston-Purina, Inc., Richmond, Ind.) and tap water ad libitum. Mice were acclimated at least 2 weeks prior to experimental use.

Drug

MVE-2 was the generous gift of Dr. David S. Breslow, Hercules, Inc. (Wilmington, Del.). The compound was dissolved in distilled water and adjusted to pH 7.2 with 1 N NaOH. A calcium salt was prepared from this stock solution using 10.5% CaCl2·2H2O, and dilutions were made with 0.15 M NaCl. The isotonic solution used for injections was composed of 0.14 N NaCl and 0.009 M CaCl2. Vehicle control solution was also prepared. All solutions were prepared fresh weekly and kept refrigerated.

Peritoneal cells were collected 7 days after an injection of MVE-2, 25 mg/kg i.p.; alveolar cells were collected 2 days after injections of 25 mg/kg i.v. on 2 consecutive days.

Tumors

The M109 spontaneously arose in the lung of a BALB/c mouse in 1964 (16) and has been maintained in our laboratory in BALB/c mice by i.m. inoculations of 5 × 10⁶ cells at biweekly intervals. A single-cell suspension was prepared by trypsinizing nonnecrotic tumor sections for 45 min at room temperature in RPMI-1640 (0.25% trypsin) containing 1% penicillin (5000 units/ml) and streptomycin (5000 μg/ml). After exposure to calf serum, tumor cells were washed twice, suspended, and counted on a Model ZBI Coulter Counter (Coulter Electronics, Hialeah, Fla.). Viability was determined using the trypan blue exclusion technique, and bacterial contamination was monitored by incubating 10⁶ tumor cells with 2 ml of brain heart infusion broth for 72 hr at 37°C.

The term “activation” is reserved for the modification of macrophage activity that results in cytotoxic effects on tumor cells.
M109 cells were grown in vitro in CMEM. The components of this medium were obtained from Grand Island Biological Co., Grand Island, N. Y.

B16 melanoma cells were maintained by serial passage in C57BL/6 mice and in vitro under conditions identical to those for M109 cells. L5178Y lymphoma cells were maintained in Fischer’s medium supplemented with 10% horse serum and passed 3 times/week to ensure continual log-phase growth.

In studies involving M109 footpad tumors, 8 x 10^5 tumor cells in 0.02 ml RPMI-1640 were injected into the rear left footpads of BALB/c mice. In certain experimental groups, the mice were anesthetized, and the tumor-bearing leg was surgically amputated using an Electrosectm cautery unit (Birchert Corp., Los Angeles, Calif.). Primary tumor removal occurred 13 days after implantation of the tumor, at which time 100% of the mice had lethal pulmonary metastases. Mice were checked daily for mortality, and mean and median survival times were calculated.

Macrophage Procurement and Characterization

Mice were sacrificed, and peritoneal cells were collected by injecting 9 ml of RPMI-1640 into the peritoneal cavity. Alveolar cells were collected following a transection of the trachea, into which was placed a sterilized polyethylene tube (PD-60; Clay-Adams, Parsippany, N. J.). Ten 1-ml washes of 0.15 M NaCl were collected per mouse. Cells were pooled from each group of mice, washed, counted, and plated in CMEM at 37°C, 5% CO2, and 95% humidity; 2 hr later, nonadherent cells were removed by repeated rinses with PBS.

Both the peritoneal and alveolar cells obtained by the above procedures were routinely characterized for the expression of macrophage characteristics. Known numbers of cells were incubated for 2 hr in 24-well tissue culture plates (Costar; Cambridge, Mass.), at which time each well was repeatedly rinsed with PBS and the remaining adherent cells were counted. The percentage of esterase-positive cells (28) was calculated.

Macrophage-mediated Tumor Cytotoxicity

In Vitro Growth. Peritoneal cells were collected from untreated or MVE-2-treated BALB/c mice, washed, counted, and plated in CMEM in wells of a 24-well plate. Concentrations were adjusted to yield 10^6 adherent cells/well after repeated rinsing with PBS following a 2-hr incubation at 37°C and 5% CO2. B16 melanoma cells in nonconfluent cultures were trypsinized, washed, and counted, and 5 x 10^6 cells were added to certain wells. At selected intervals after cocultivation, the wells of one plate were washed with PBS and Ca2+- and Mg2+-free Hanks’ balanced salt solution. Trypsin-EDTA was added to each well, and trypsin-sensitive, nonadherent cells were collected and counted. Remaining plates were rinsed free of any nonadherent cells and fed daily with CMEM. The number of cells removed by trypsin in wells containing macrophages (Mφ) alone (consistently 5% of total cells plated) was subtracted from the number of trypsin-sensitive cells removed from the wells containing both resident (RES) or MVE-2-activated Mφ and B16 melanoma cells. These values, B16RES and B16MVE, respectively, were compared to the number of trypsin-sensitive B16 cells growing in the absence of macrophages.

Cytolysis Assay. B16 or M109 cells in their log phase of growth in vitro were labeled overnight with CMEM containing 1 μCi of [3H]TdR per ml (10 Ci/mmol; Amersham Corp., Arlington Heights, Ill.). Tumor cells were collected after exposure to trypsin-EDTA for 6 min at 37°C, washed twice with CMEM, and resuspended at a concentration of 5 x 10^6 trypan blue-excluding cells per ml of CMEM. About 10^6 target cells were added to varying numbers of adherent cells in wells of a microtiter plate (Microtest II; Falcon Products, Oxnard, Calif.). Adherent cells were enumerated to obtain precise values for E:T cell ratios.

Cytostasis Assay. Cytostatic activity was determined with the same cells used in the cytotoxic assay. After the supernatant fluid was collected, the cells were repeatedly rinsed with PBS and incubated with 0.25 μCi of [3H]TdR (2000 Ci/mmol; New England Nuclear, Boston, Mass.) in fresh CMEM at 37°C for 6 hr. The supernatant was removed, and the cells were repeatedly rinsed with PBS, fixed with methanol, and sprayed with a lacquer aerosol. The microtiter plate was cut into individual wells with a bandsaw, and uptake of [3H]TdR was counted in a Beckman liquid scintillation counter.

Cloning Assay. Alveolar or peritoneal cells were collected, washed, and plated in wells of a 24-well plate. Concentrations were adjusted to yield 10^6 adherent cells/well after repeated rinsing with PBS following a 2-hr incubation at 37°C and 5% CO2. B16 melanoma cells in nonconfluent cultures were trypsinized, washed, and counted, and 5 x 10^6 cells were added to certain wells. At selected intervals after cocultivation, the wells of one plate were washed with PBS and Ca2+- and Mg2+-free Hanks’ balanced salt solution. Trypsin-EDTA was added to each well, and trypsin-sensitive, nonadherent cells were collected and counted. Remaining plates were rinsed free of any nonadherent cells and fed daily with CMEM. The number of cells removed by trypsin in wells containing macrophages (Mφ) alone (consistently 5% of total cells plated) was subtracted from the number of trypsin-sensitive cells removed from the wells containing both resident (RES) or MVE-2-activated Mφ and B16 melanoma cells. These values, B16RES and B16MVE, respectively, were compared to the number of trypsin-sensitive B16 cells growing in the absence of macrophages.
counted, and incubated at varying concentrations in 24-well plates at 37° and 5% CO₂ for 2 hr, at which time nonadherent cells were rinsed off. Nonadherent L5178Y cells were added to selected wells and incubated for 48 hr. Tumor cells were removed, and 500 cells were plated in 100-mm-diameter tissue culture dishes in Fisher's medium containing 10% horse serum and 10% Noble agar. Colonies of surviving tumor cells were counted 18 days later using an Artek Model 980 colony counter (Arlington, Va.).

Statistical Analysis

The level of confidence for all experiments was set at 95%. A one-way analysis of variance with Dunnett's t test was used when one control group was compared to more than one experimental group (1). Linear regression was used to determine the slopes of growth curves. Differences in median survival time were assessed by the Wilcoxon rank sum test (26).

RESULTS

Tumoristant and Tumoricidal Activity of MVE-2-activated Macrophages. The cytotoxicity of MVE-2-activated macrophages was tested against 3 tumor target cells, using both growth inhibition and cytotoxicity assays. The growth of B16 melanoma cells in the presence of peritoneal macrophages collected from BALB/c mice treated 7 days earlier with MVE-2 was markedly inhibited, ranging from 78% inhibition after 4 hr to 85% inhibition after 65 hr of cocultivation (Chart 1). Resident peritoneal macrophages had no effect upon the number of B16 tumor cells growing in vitro.

When M109 carcinoma and B16 melanoma cells were used as targets in a [³H]dThd release assay, MVE-2-activated peritoneal macrophages had a cytolytic activity of up to 20% against the M109 cells and up to 87% against the B16 cells (Table 2). Resident macrophages had little or no significant cytolytic activity against either target cell. Macrophages from treated or untreated mice possessed significant cytoytic activity against both target cells. The higher MVE macrophage E:T ratio groups inhibited the uptake of [¹²⁵]I]dUrd by M109 and B16 tumor cells by 99 and 98%, respectively, and exhibited significantly greater cytolytic and cytoytic activity compared to resident macrophages. Similar results were obtained using peritoneal macrophages harvested from untreated and MVE-2-treated C57BL/6 mice (data not shown).

In order to activate macrophages in the pulmonary metastatic bed, MVE-2 was administered i.v. Alveolar macrophages recovered by lung lavage from mice given MVE-2 i.v. possessed a 36% cytolytic and 96% cytoytic activity against M109 cells, compared to a 34 and 95% activity, respectively, by peritoneal macrophages activated by MVE-2 administered i.p. (Table 3). Also, the cytoytic and cytoytic activity of alveolar macrophages from MVE-2-treated mice were significantly higher than the activities observed with resident macrophages at identical E:T ratios. Incubation of B16 target cells with alveolar macrophages from mice receiving MVE-2 i.v. released nearly all the radioactivity (i.e., 99% cytoytic activity; data not shown).

A cloning assay also demonstrated the tumoricidal and tumoristant activity of macrophages isolated from mice receiving MVE-2 (Table 4). Following a 2-day incubation with alveolar or peritoneal macrophages, L5178Y lymphoma cells were removed, plated in agar, and examined 18 days later for colony formation. Alveolar macrophages recovered from mice receiving MVE-2 i.v. significantly inhibited the growth of the lymphoma cells at an E:T ratio of 50:1. As also found previously (12), MVE-2-activated peritoneal macrophages were inhibitory at an E:T ratio of 10:1.

Therapeutic Efficacy of MVE-2 against M109. Based on the results of the in vitro cytotoxicity assays, experiments were designed to determine if mice bearing the lung-metastasizing M109 would show an ILS following MVE-2 administration i.v., using the same dosing schedule which resulted in tumoricidal alveolar macrophages in vitro (Table 5). One week after footpad implantation of 8x10⁵ M109 cells, MVE-2 was injected i.v. for 2 consecutive days. Certain groups received this 2-day injection schedule for a total of 4 weeks. Primary tumor amputation was performed on one-half of the animals to determine if immunotherapy combined with surgery was more effective than either treatment alone. Weekly injections of MVE-2 given 2 days in succession resulted in a significant ILS over vehicle-treated controls in animals bearing their primary tumor and in mice undergoing surgical amputation. Weekly injections were superior to a single 2-day injection schedule. In mice bearing primary tumors, an ILS, based on mean survival time, of 31 and 32% for weekly injection was observed, compared to 13 and 11% (MVE-2, 25 and 50 mg/kg, respectively) for a single 2-day injection schedule. For animals whose primary tumor was removed, weekly injections increased the life span from 13 to 46% (single 2-day injections) to 57 and 74% (MVE-2, 25

Chart 1. Growth of B16 melanoma cells in vitro in the presence of resident or MVE-2-activated peritoneal macrophages. Peritoneal cells from resident or MVE-2 (25 mg/kg i.p., Day 7)-treated BALB/c mice were purified by adherence after a 2-hr incubation. B16 melanoma target cells were added at an E:T ratio of 20:1. At various intervals, trypsin-sensitive cells were removed and counted, while remaining cultures were washed and fresh CMEM was added. The number of cells removed by trypsin in wells containing macrophages alone was subtracted from the number of trypsin-sensitive cells removed from the wells containing both resident or MVE-2-activated macrophages and B16 melanoma cells. These values, B16RES and B16MVE-2, respectively, were compared to the number of trypsin-sensitive B16 cells growing in the absence of macrophages. Points, mean number of cells derived from quadruplicate cultures.

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Cytotoxicity of BALB/c peritoneal macrophages against M109 carcinoma and B16 melanoma target cells following MVE-2 administration

Macrophages from resident or MVE-2 (25 mg/kg i.p., Day −7)-treated mice were collected and monitored for cytolytic and cytostatic activity.

<table>
<thead>
<tr>
<th>Group</th>
<th>E:T ratio</th>
<th>[3H]TdTh released (cpm)</th>
<th>Cytolytic activity (%)</th>
<th>[3H]dUrd uptake (cpm)</th>
<th>Cytostatic activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M109 alone</td>
<td>15:1</td>
<td>10,701 ± 224²</td>
<td>5</td>
<td>4,765 ± 401²</td>
<td>63</td>
</tr>
<tr>
<td>Resident macrop</td>
<td>18:1</td>
<td>15,343 ± 513</td>
<td>3</td>
<td>296 ± 40²</td>
<td>99</td>
</tr>
<tr>
<td>MVE macrop</td>
<td>18:1</td>
<td>18,937 ± 360</td>
<td>6</td>
<td>5,899 ± 1,028²</td>
<td>66</td>
</tr>
<tr>
<td>B16 alone</td>
<td>18:1</td>
<td>14,147 ± 550</td>
<td>11</td>
<td>10,164 ± 1,523²</td>
<td>36</td>
</tr>
<tr>
<td>Resident macrop</td>
<td>18:1</td>
<td>18,859 ± 1,838</td>
<td>11</td>
<td>10,164 ± 1,523²</td>
<td>36</td>
</tr>
<tr>
<td>MVE macrop</td>
<td>18:1</td>
<td>52,150 ± 6,755</td>
<td>87</td>
<td>5,399 ± 1,028²</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>24:1</td>
<td>50,850 ± 1,297</td>
<td>84</td>
<td>407 ± 190²</td>
<td>95</td>
</tr>
</tbody>
</table>

² Maximum release, 39,277 ± 273.
³ Mean ± S.E.
+p < 0.05 compared to target cell alone.
+p < 0.05 compared to resident macrophages.
Maximum release, 58,807 ± 2,264.

Table 3
Antitumor activity of alveolar and peritoneal macrophages against M109 following MVE-2 administration

Alveolar and peritoneal macrophages were collected from BALB/c mice following i.v. and i.p. administrations of MVE-2, respectively. Resident macrophages were collected from untreated animals and all groups were monitored for cytolytic and cytostatic activity.

<table>
<thead>
<tr>
<th>Group</th>
<th>E:T ratio</th>
<th>[3H]TdTh released (cpm)</th>
<th>Cytolytic activity (%)</th>
<th>[3H]dUrd uptake (cpm)</th>
<th>Cytostatic activity (%)</th>
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<td>63</td>
</tr>
<tr>
<td>Resident macrop</td>
<td>18:1</td>
<td>15,343 ± 513</td>
<td>3</td>
<td>296 ± 40²</td>
<td>99</td>
</tr>
<tr>
<td>MVE-2 i.v.</td>
<td>18:1</td>
<td>18,937 ± 360</td>
<td>6</td>
<td>5,899 ± 1,028</td>
<td>66</td>
</tr>
<tr>
<td>MVE macrop</td>
<td>18:1</td>
<td>52,150 ± 6,755</td>
<td>87</td>
<td>5,399 ± 1,028</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>24:1</td>
<td>50,850 ± 1,297</td>
<td>84</td>
<td>407 ± 190²</td>
<td>95</td>
</tr>
</tbody>
</table>

³ Mean ± S.E.
+p < 0.05 compared to tumor alone.
+p < 0.05 compared to resident macrophages.

Table 4
L5178Y colony formation following incubation with macrophages harvested from BALB/c mice exposed to MVE-2

Alveolar macrophages were collected from untreated or MVE-2-treated mice, incubated for 2 days with L5178Y cells. Tumor cells were removed and scored after 18 days for colony formation. Peritoneal cells were collected and tested in an earlier experiment.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>E:T ratio</th>
<th>Colony inhibition (%)</th>
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</thead>
<tbody>
<tr>
<td>Alveolar macrop</td>
<td>25:1</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>50:1</td>
<td>0</td>
</tr>
<tr>
<td>MVE-2 i.v. b</td>
<td>25:1</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>50:1</td>
<td>37²</td>
</tr>
<tr>
<td>Peritoneal macrop</td>
<td>10:1</td>
<td>54 c</td>
</tr>
</tbody>
</table>

² % of inhibition = [1 - (tumor + macrophage)/tumor]100.
b Mice received 25 mg/kg on 2 consecutive days prior to harvesting 2 days later.
p < 0.05 compared to colony formation by L5178Y not incubated with macrophages.
Mice received 25 mg/kg 1 week prior to harvesting.

and 50 mg/kg, respectively). Surgery alone did not significantly prolong survival time, but when coupled with MVE-2 treatment, either on Days 7 and 8 or weekly thereafter, substantial increases in median survival times were seen when compared to identically dosed, nonamputated groups. In groups given 50 mg of MVE-2 per kg, an ILS of 9% following 2 doses of MVE-2 was increased to 78% if the primary tumor was subsequently removed on Day 13. When weekly injections were administered, the ILS went from 34% (nonamputated) to 85% following primary tumor removal. All mice were autopsied and examined for the presence of macroscopic metastatic tumor. The majority of animals had from 25 to 35 small tumor nodules on the surfaces of the lung. In contrast, certain drug-treated animals dying 60 days or more after footpad inoculation presented lungs with 1 to 3 larger nodules surrounded by normal lung tissue. The lung of one animal was macroscopically free of tumor, but 2 or 3 metastatic liver tumor foci were found to be present. Liver tumor foci were not observed in any other mice.
Tables

Survival time of BALB/c mice bearing M109 following i.v. administration of MVE-2

BALB/c mice were given injections of 8 × 10⁶ M109 cells in the left hind footpad; 1 week later all mice were randomized and treatment commenced. On Day 13, the primary tumor was surgically excised from animals in selected groups. Group 1, drugs administered on Days 7 and 8. Group 2, drugs administered on Days 7, 8, 14, 15, 21, 22, 28, and 29.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>Median survival time (days)</th>
<th>Mean survival time (days)</th>
<th>ILS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No surgery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1 Vehicle</td>
<td>16</td>
<td>35.5</td>
<td>35.9 ± 1.6c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVE-2</td>
<td>25</td>
<td>8</td>
<td>35.5</td>
<td>38.9 ± 2.2</td>
<td>13</td>
</tr>
<tr>
<td>MVE-2</td>
<td>50</td>
<td>8</td>
<td>37.0</td>
<td>38.1 ± 2.6</td>
<td>11</td>
</tr>
<tr>
<td>Group 2 Vehicle</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>MVE-2</td>
<td>25</td>
<td>8</td>
<td>35.5</td>
<td>36.5 ± 2.0</td>
<td>31</td>
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<tr>
<td>MVE-2</td>
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<td>8</td>
<td>47.5</td>
<td>47.8 ± 2.0a</td>
<td>32</td>
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<td>Day 13 amputation</td>
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<td>Surgery control</td>
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<tr>
<td>Vehicle</td>
<td>15</td>
<td>34.0</td>
<td>39.6 ± 4.0</td>
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<tr>
<td>MVE-2</td>
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<td>8</td>
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<td>42.9 ± 5.0</td>
<td>13 (1)*</td>
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<tr>
<td>MVE-2</td>
<td>50</td>
<td>7</td>
<td>57.0</td>
<td>55.4 ± 7.3</td>
<td>46</td>
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<tr>
<td>Group 2 Vehicle</td>
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<td>MVE-2</td>
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<td>32.5</td>
<td>33.8 ± 4.0</td>
<td>57</td>
</tr>
<tr>
<td>MVE-2</td>
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<td>7</td>
<td>56.5</td>
<td>53.2 ± 7.2b</td>
<td>57</td>
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<tr>
<td>MVE-2</td>
<td></td>
<td></td>
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</tbody>
</table>

a As defined in "Materials and Methods."
b Compared to appropriate vehicle control animals.
c Mean ± S.E. of animals which died.
d p < 0.05 by Wilcoxon rank sum (median) or analysis of variance with Dunnett's t test (mean of dead animals).

DISCUSSION

An overall goal in this laboratory has been to modulate the distribution of MVE-2 so that macrophages and tumor cells are in contact with one another, an apparent prerequisite for cytostasis and tumor cell damage (7, 10). A number of different studies, in which the biological immunomodulators C. parvum and BCG induced cytotoxic alveolar macrophages, suggested that MVE-2 might also activate alveolar macrophages in the mouse. Murine alveolar cells collected after i.v. injections of C. parvum were reported to inhibit DNA synthesis in leukemia cells (19), while hamster alveolar macrophages were shown to be tumoricidal in vitro following intratracheal instillation of BCG (30). Scott and Decker (25) recently reported that alveolar macrophages, isolated from mice 4 days after an intrapleural injection of C. parvum, inhibited the uptake of [³H]dThd by leukemia cells. In addition, mice given C. parvum intrapleurally prior to i.v. injections of T3 fibrosarcoma cells had a mean survival time twice that of mice not receiving intrapleural administration of C. parvum.

The ability of MVE-2 to induce a population of tumoricidal alveolar macrophages and increase the life span of M109-bearing mice appeared to depend not only upon the route of administration but also on the schedule. Two successive doses of MVE-2 by the i.v. route of administration were necessary to induce cytotoxic alveolar macrophages; this same dosing regimen resulted in significant increases in both median and mean survival times of M109-inoculated mice compared to vehicle controls. The rationale for this schedule came primarily from studies in which the functional status of the reticuloendothelial system was determined after i.v. administration of the parent MVE copolymer (18). In these studies, 2 i.v. injections of MVE were given 2 days in succession. One day later, a marked prolongation of intravascular clearance of ⁵¹Cr-labeled sheep RBC was observed, which was highly correlated with a 78% decrease in uptake of sheep RBC by the liver. Although there are no data at the present time concerning reticuloendothelial system status and MVE-2 distribution following a second MVE-2 i.v. dose, the possibility exists that the Kupffer cells of the liver are compromised in their ability to clear the second injection from the blood, thereby increasing the concentration of MVE-2 in the peripheral blood and the metastatic tumor bed. The possibility of a direct cytotoxic effect on tumor cells in vivo appears unlikely, due to the wide difference in the amount of drug injected and the amount required for inhibition of M109 cells during continuous drug exposure in vitro.

Increasing the concentration of MVE-2 in the blood and lung not only may activate alveolar macrophages but may also affect another antitumor cell type, the NK cell. Significant enhancement of activity in NK cells isolated from spleen and disaggregated lung tissue was detected in mice given i.v. injections of MVE 3 days prior to testing (20). When 3-week-old nude mice exhibiting low levels of NK activity received either of 2 other biological response modifiers, polyinosinic-polycytidylic acid or C. parvum, marked increases in NK activity were detected 24 hr later (4). More importantly, if tumor cells were injected i.v. 1 day after drug treatment, a significant decrease in the...
number of experimental pulmonary metastases was observed (5).

The possibility remains, therefore, that M109 cells with metastatic potential, which are known to be in systemic circulation by Day 7 postimplantation, may encounter activated NK cells, as well as activated alveolar macrophages, following MVE-2 treatment on Days 7 and 8. Since NK cells appear to play a role in the inhibition of tumor metastases (5, 20) and activated macrophages are capable of impeding the growth of already established lung metastases (2, 14), the beneficial effect on survival of M109-bearing mice receiving MVE-2 may be due to an effect on both cell types, each with its own kinetics of activation. The observation that MVE can both augment and suppress NK activity, presumably via macrophage-NK cell interactions (21, 22), illustrates the need for further studies to discern the contributions of each of these antitumor effector cells in controlling metastatic growth.

In conclusion, these studies are the first to demonstrate that alveolar macrophages from mice given MVE-2 are cytotoxic to tumor cells in vitro. The results of these experiments, coupled with results of ongoing MVE-2 distribution studies, will be used to determine an optimal dosing regimen for tumor-bearing hosts. Utilization of a treatment plan involving the activation of both alveolar macrophages and NK cells, in combination with other anticancer modalities, may well result in a more effective therapeutic approach to metastatic lung disease.

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REFERENCES

Maleic Vinyl Ether Activation of Murine Macrophages against Lung-metastasizing Tumors

Scott E. Loveless and Albert E. Munson