Involvement of Macrophages in the Eradication of Established Metastases following Intravenous Injection of Liposomes Containing Macrophage Activators


ABSTRACT

Liposomes containing encapsulated lymphokines or muramyl dipeptide (MDP), when injected i.v. into C57BL/6 mice, produce significant destruction of established lung and lymph node metastases from a s.c. highly metastatic B16-BL6 melanoma. We present evidence that eradication of the metastases is mediated by the activation of host macrophages to the tumoricidal state. Results from three separate types of experiments support this conclusion. (a) When macrophage-activating agents such as lymphokines or MDP were delivered in liposomes that were not efficiently retained in the lung, little or no activation of lung macrophages was observed, and growth of metastases was unaltered. (b) Eradication of metastases was not observed when tumor-bearing animals were treated with agents that impaired macrophage function (e.g., silica, carrageenan, hyperchlorinated drinking water) prior to systemic therapy with liposome-encapsulated lymphokines or liposome-encapsulated MDP. (c) Macrophages activated in vitro by liposome-encapsulated MDP and then injected i.v. into mice bearing experimental lung metastases also significantly inhibited lung metastases. These results suggest that the augmented host response against pulmonary and lymph node metastases generated by the systemic administration of liposome-encapsulated lymphokines or MDP is mediated via activated cytotoxic macrophages.

INTRODUCTION

Recent studies from our laboratories have shown that systemic administration of lymphokines (13) or MDP (17) encapsulated in liposomes leads to the activation of tumoricidal properties in macrophages and is associated with destruction of established pulmonary and lymph node metastases originating from a s.c. melanoma. The mechanism(s) by which these modalities induce tumor destruction is not clear. The evidence for the role that macrophages play in the destruction of metastases is circumstantial and comes from studies showing that liposomes containing immunomodulators can produce efficient activation of tumoricidal properties in macrophages both in vitro (35, 36) and in vivo (19). The possibility, however, that macrophage activation and tumor destruction are independent events cannot be excluded, nor can we rule out the possibility that effector cells other than macrophages are responsible for the destruction of metastases following the systemic administration of liposomes containing lymphokines or MDP. For example, in addition to containing a lymphokine that activates macrophages, the unpurified lymphokine preparations used in these experiments contain mediators that affect a wide range of specific and nonspecific host defense reactions. Similarly, MDP, although highly effective in stimulating macrophages (17, 43), also affects lymphocyte-mediated cellular and humoral immune reactions (7, 8, 28, 34). In the present report, we present evidence that the macrophage is the essential host cell responsible for the destruction of metastases in animals treated with either lymphokines or MDP encapsulated in liposomes.

MATERIALS AND METHODS

Animals. Specific-pathogen-free C57BL/6 mice, 6 to 8 weeks old, and nude mice (BALB/c background), 3 to 4 weeks old, were obtained from the Frederick Cancer Research Facility Animal Production Area, the West Seneca Animal Breeding Facility of Roswell Park Memorial Institute, and the Central Laboratory Animal Service Division of Smith, Kline and French Laboratories.

Cell Cultures. The B16-BL6 variant line of the C57BL/6 melanoma has been adapted to grow in vitro. Following implantation at a s.c. site, this tumor metastasizes to the lungs and lymph nodes in about 90% of mice (22). All monolayer cultures were maintained in Eagle's minimal essential medium supplemented with 5% fetal bovine serum, vitamin solution, sodium pyruvate, nonessential amino acids, and L-glutamine. The components of this medium were obtained from Flow Laboratories, Rockville, Md. The medium was endotoxin-free as determined by the Limulus amebocyte lysate assay (Associates of Cape Cod, Woods Hole, Mass.). All cultures were incubated at 37° in a humidified atmosphere containing 5% CO2. All cell cultures were free of Mycoplasma, reovirus type 3, pneumonia virus of mice, K virus, Theliers encephalomyelitis virus, Sendai virus, minute virus of mice, mouse adenovirus, mouse hepatitis virus, lymphocytic choriomeningitis virus, ecmatoma virus, and lactate dehydrogenase virus (assayed by M. A. Bioproducts, Walkersville, Md.).

Preparation and Purification of AM Cultures. Mouse AMs were harvested by a tracheobronchial lavage method described fully elsewhere (24). The lavaged AM suspension was centrifuged and resuspended; 10⁶ AMs were plated into wells of a Microtest II plate with a surface area of 38 sq mm (Falcon Plastics, Oxnard, Calif.) in serum-free media. Nonadherent cells (fewer than 10%) were removed by washing with media 60 min after initial plating. At that time, practically all (>91%) of the adherent cells had a mononuclear morphology, phagocytosed carbon particles, and/or opsonized sheep RBC (20). Lymphokines. Cell-free supernatants were harvested from mitogen-
stimulated rat lymphocytes and from unstimulated control lymphocytes as described previously (37). MDP. MDP was purchased from Calbiochem-Behring Corp., La Jolla, Calif. The MDP did not contain endotoxins as detected by the *Limulus* amebocyte lysate assay.

**Lipids and Preparation of Liposomes.** Chromatographically pure egg PC and beef brain PS were purchased from Avanti Biochemicals, Inc., Birmingham, Ala. Liposomes were prepared from a mixture of PC and PS (7:3 mol ratio) or PC alone and were mechanically agitated on a vortex mixer to form multimamellar vesicles as described elsewhere (36). Lymphokines, MDP, or HBSS were encapsulated within liposomes by methods similar to those described previously (17, 43). PC:PS liposomes were used because they are not toxic at the dose used here (2.5 μmol phospholipid per mouse) and, following i.v. injection, they are arrested efficiently in the lungs (in addition to organs of the RE system) (16, 23). Liposome preparations were always used within 4 hr. The internal volume of the liposomes used here was determined to be 2.5 ± 0.3 (S.D.) μl/μmol phospholipid (36). This dose of liposomes contained approximately 6.25 μl encapsulated material (HBSS, lymphokines, or MDP).

**Activation of AM following i.v. Injection of Free and/or Liposome-encapsulated Materials.** Mice were given i.v. injections of HBSS (0.2 ml), lymphokines (200 μl), free MDP (200 μg/mouse), or liposomes (2.5 μmol phospholipids) containing HBSS, lymphokines, or MDP (2.5 to 5 μg). The liposomes were suspended in HBSS in a volume of 0.2 ml. Liposomes containing HBSS and suspended in a mixture of 0.2 ml HBSS and unencapsulated (free) MDP (6.25 μg/mouse) or lymphokines (6.25 μl) were used as controls. Mice were killed 24 hr after the i.v. injection, and their AMs were harvested by lavage.

**Macrophage-mediated Cytotoxicity Assay.** Macrophage-mediated cytotoxicity was assessed by a radioactive release assay as described previously (37). AM cultures were washed with media, and 5 × 10^5 [125I]iododeoxyuridine-labeled target cells were added to each well in 0.2 ml medium containing 5% fetal bovine serum. Target cells alone were always plated as an additional control. Twenty-four hr after the addition of target cells, the cultures were washed and re-fed to remove nonplated cells. Adherent target cells were lysed with 0.1 ml of 0.2 N NaOH at 24 or 72 hr after plating. The lysate was absorbed on cotton swabs, placed directly into 12- × 75-mm tubes, and monitored for radioactivity in a gamma counter. The percentage of cytotoxicity in the macrophage assays was computed by the following formula:

% of cytotoxicity =

\[
\frac{\text{cpm of target cells with control macrophages} - \text{cpm of target cells with activated macrophages}}{\text{cpm of target cells with control macrophages}} \times 100
\]

The statistical significance of differences between groups was determined by Student’s 2-tailed t test.

**Treatment of Spontaneous Metastases by the i.v. Injection of Liposomes Containing Lymphokines or MDP.** C57BL/6 mice were given s.c. injections in the footpad of 5 × 10^5 viable B16-BL6 tumor cells suspended in a volume of 0.05 ml of HBSS. Four to 5 days later, when the tumors reached 10 to 15 mm in diameter, the mice were anesthetized by methoxyflurane inhalation, and the tumor-bearing leg, including the popliteal lymph node, was amputated at the midfemur. Three days later, mice were given injections in the tail vein of liposomes (2.5 μmol phospholipid) suspended in 0.2 ml HBSS. The liposomes, prepared from either PC alone or PC and PS at a 7:3 mol ratio, contained MDP (2.5 μg/mouse), lymphokines (6.25 μl), or HBSS. Liposomes containing HBSS were suspended in HBSS and free MDP (2.5 μg/mouse) or in 6.25 μl of unencapsulated lymphokine preparation. An additional control group included mice given i.v. injections of HBSS only. The animals were treated twice weekly for 4 weeks and were killed 2 weeks after the final treatment. Pulmonary metastases in each animal were counted under a dissecting microscope by 2 independent observers. Suspected pulmonary metastases were confirmed by microscopic examination of fixed histological sections. The results were analyzed with the χ² test.

**Measurement of the RE System Function.** The phagocytic activity of the RE system was measured by determining the rate of clearance of colloidal carbon and of 51Cr-labeled sheep RBC. Mice were weighed and lightly anesthetized with sodium pentobarbital and were given i.v. injections of 0.01 ml of a solution of colloidal carbon particles (C11/1431a; Gunther Wagner, Hanover, Germany) suspended at 25 mg/ml in sterile phosphate-buffered saline (0.2 g/l KCl, 8.0 g/l NaCl, 0.2 g/l, KH₂PO₄, 2.16 g/l NaH₂PO₄, 7H₂O) containing 1% gelatine. Blood samples (20 μl) were obtained from the orbital sinus 3 and 15 min after injection and were diluted in 4.1 ml of 0.1% Na₂CO₃. The absorbance of the samples was then measured in a Lumatron Colorimeter (Photovolt Corp., New York, N. Y.) with a 650 nm red filter to determine the concentration of carbon in the peripheral blood at the indicated times, and the phagocytic index was calculated by the equation (4).

\[ K = \log_{10} A(3 \text{min}) - \log_{10} A(15 \text{min}) / 12 \]

where A = absorbance and K = phagocytic index. A corrected value, α, for K is then corrected for differences in body and organ weight as follows:

\[ \alpha = K \times (\text{liver + spleen weight})/\text{body weight} \]

Sheep RBC were labeled with Na₂⁵¹CrO₄, as described previously (18) and injected i.v. into mice (4 × 10⁶ cpm/mouse). The radioactivity present in the peripheral blood was measured at 5 and 25 min after injection in samples obtained from the orbital sinus. Mice were killed after the second blood sample was taken, and the amount of radioactivity present in the liver and spleen was measured and expressed as a percentage of the total radioactivity injected.

**Suppression of Macrophage Function in Situ by Silica and Carrageenan.** Carrageenan and silica were titrated to establish the dosage necessary for producing >70% suppression of RE function as determined by clearance of colloidal carbon described above. Suppression of RE function persisted for 4 to 6 days and could be sustained for a further 4 to 6 days by another treatment cycle 5 days after completion of the previous treatment protocol. Silica particles (quartz particles; average particle diameter, 2.8 μm; Whittaker, Clark, and Daniels, Inc., South Plainfield, N. J.) were sonicated in sterile phosphate-buffered saline immediately before use. Mice were given i.v. injections of 2.5 μg silica in 0.2 ml phosphate-buffered saline, and 4 hr later they were given i.p. injections of 25 mg silica in 1.5 ml phosphate-buffered saline; treatment was then repeated 48 hr later. The i.v. and i.p. injections of silica were combined because i.v. injection alone produces only limited impairment of peritoneal macrophages (29).

Unfractionated carrageenan, obtained from Marine Colloids, Inc., Rockland, Maine, was dissolved in HBSS at a concentration of 1 mg/ml. Mice were given a total of 2 i.v. injections (0.2 mg/mouse) at 2-day intervals. By monitoring the carbon clearance from treated animals, we were able to establish that this procedure suppressed RE activity for 4 to 5 days and that a second treatment initiated 5 days after the first was effective in maintaining suppressive activity.

**Suppression of Macrophage Activity in Situ by Administration of Hyperchlorinated Drinking Water.** Four-week-old female C57BL/6N mice were given sterile tap water (1.0 ppm chlorine) or hyperchlorinated drinking water (25 ppm chlorine) (12). The chlorine and chloramine content were assayed by the o-toluidine method by which total chlorine is measured (Cl²⁻, HOCl, NH₂Cl, NHCl₂, and NCl₃) (41). Chlorinated drinking water was prepared twice weekly by adding a solution of sodium hypochlorite to tap water. Four weeks after this treatment began, mice were given i.p. injections of 2 ml of thiglycollate broth (Baltimore Biological Laboratories, Cockeysville, Md.). Five days later, peritoneal macrophages were collected and counted. The ability of these PEC to phagocyte ⁵¹Cr-labeled sheep RBC was determined by techniques described previously (18). The ability of macrophages in mice given tap or hyperchlorinated water to respond to activation
stimuli in vivo was also examined. Mice were given i.v. injections of HBSS or liposomes containing either MDP (2.5 μg/mouse) or HBSS. Twenty-four hr later, AMs were harvested, and their in vitro cytotoxicity was assayed. Spontaneous B16-BL6 metastases in mice given tap or hyperchlorinated water were treated by the procedure described above.

Adaptive Transfer of Macrophages Activated in Vitro. PEC were harvested from thioglycollate-stimulated normal C57BL/6 mice as described above. The PEC were plated in serum-free medium onto plastic dishes for 40 min. All nonadherent cells were then removed, and the adherent PEC were gently scraped off the dish with a rubber policeman. Plating for 40 min in serum-free medium minimum essential medium preferentially selects for macrophages since practically all the PEC incubated in suspension as described above were phagocytic and exhibited a morphology typical of macrophages (37). These PEC were suspended in media containing 1 μmol of PC:PS liposome-encapsulated MDP (1 μg) per 10^6 PEC per ml. Control cultures consisted of macrophages incubated with liposomes containing HBSS but suspended in free MDP (1 μg/ml). The PEC-liposome suspensions were placed into polypropylene test tubes and kept on a rocking platform for 16 hr at 37°. Following this incubation, the macrophage suspensions were centrifuged and resuspended in HBSS. One portion of the macrophages was used for in vitro assays to measure cytotoxicity, and the other portion was injected i.v. into metastasis-bearing syngeneic mice.

To produce metastases in C57BL/6 mice, B16-BL6 cells grown in vitro were harvested during their exponential growth phase by a 1-min treatment with 0.25% trypsin-0.02% EDTA. The cells were washed twice with media and then were resuspended in HBSS. The number of single viable tumor cells (>90% viability, trypan blue exclusion test) was determined. Twenty thousand tumor cells were injected i.v. into the tail veins of normal mice. Three days later, when parenchymal micrometastases were established (11), treatment with macrophages was used for in vitro assays to measure cytotoxicity, and the other portion was injected i.v. into metastasis-bearing syngeneic mice.

Effect of Antimacrophage Agents on Host Response to Liposome-encapsulated Lymphokines and MDP. To test the in situ activation of tumoricidal properties in AMs and destruction of spontaneous pulmonary metastases by i.v. injection of liposomes containing MDP or lymphokines, groups of 3 mice were given i.v. injections of the indicated materials 24 hr before AMs were harvested by pulmonary lavage. Liposomes (2.5 μmol lipid per mouse) were suspended either in HBSS or in HBSS containing lymphokines or MDP. Twenty-four hr after addition of target cells, and cell-associated radioactivity was measured after 72 hr. The results are mean percentages of cytotoxicity from triplicate cultures.

Results

Activation of AMs and Destruction of Pulmonary Metastases by Systemic Administration of Lymphokines or MDP Encapsulated in Liposomes. Liposomes (PC:PS, 7:3 mol ratio) containing encapsulated lymphokines or MDP, when injected i.v. into C57BL/6 mice, bring about a significant reduction in the number of lung metastases from a primary B16 melanoma tumor growing s.c. In addition to stimulating tumor destruction, these liposomes activate AMs and render them cytotoxic to tumor cells in vitro (Table 1). In contrast, the i.v. injection of MDP or lymphokines encapsulated in PC liposomes does not lead to the activation of tumoricidal properties in AMs or to a reduction in lung metastases (Table 1). PC liposomes containing lymphokines can activate AMs or peritoneal macrophages following in vitro incubation. PC liposomes, however, are not arrested in the lung vasculature as efficiently as are PC:PS liposomes (16). We suggest, therefore, that lymphokines or MDP encapsulated in PC liposomes do not activate AMs in situ to enhance tumor destruction simply because liposomes of this composition are unable to deliver adequate amounts of the activating agents to the macrophages. In any event, these data demonstrate a good correlation between the ability of the injection preparation to activate tumoricidal properties in AMs and inhibition of lung metastasis.

Effect of Antimacrophage Agents on Host Response to Liposome-encapsulated Lymphokines and MDP. To test
whether tumoricidal macrophages are responsible for the enhanced destruction of metastases produced by treatment with PC:PS liposomes containing encapsulated lymphokines or MDP, we administered agents that impair macrophages in situ to animals immediately before and after liposome therapy.

Treatment of tumor-bearing mice with silica, carrageenan, or hyperchlorinated drinking water produces a significant reduction in the recovered number of AMs or PEC. Moreover, these agents impair the in situ phagocytic activity of macrophages (Table 2). These effects are not peculiar to tumor-bearing animals, however, because identical adverse effects on macrophages occur in normal animals subjected to these treatments (data not shown). In addition to reducing the macrophage population and phagocytic activity, the treatments increase the incidence of metastasis and destroy the ability of liposome-encapsulated lymphokines or MDP to activate AM in situ and to produce destruction of established lung metastases (Table 3). These results suggest that the enhanced host resistance to metastases produced by treatment with lymphokines or MDP encapsulated in liposomes is mediated by activated macrophages.

Effect of Adoptive Transfer of Macrophages Activated in Vitro on Growth of Lung Metastases. Additional evidence suggesting that macrophages activated by liposome-encapsulated MDP can act directly as cytotoxic effector cells and probably represent the major effector mechanism responsible for the augmented destruction of metastases seen in animals given injections of liposome-encapsulated MDP has been obtained in adoptive transfer experiments. For these experiments, we used mice bearing experimentally induced pulmonary metastases. These recipients were treated with multiple i.v. injections of macrophages preincubated in vitro with liposomes containing either HBSS (controls) or MDP. Macrophages incubated in vitro with liposomes containing MDP were cytotoxic to tumor targets in vitro: when they are injected i.v. into mice bearing experimental metastases, the number of lung tumor colonies was significantly reduced (Table 4). In contrast, adoptive transfer of macrophages incubated in vitro with empty liposomes containing 0.9% NaCl solution and free MDP had no significant effect on metastatic burden (Table 4).

**DISCUSSION**

In this paper, we present several lines of evidence to support our conclusion that the eradication of established spontaneous metastases in mice following the systemic administration of

Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>In vivo phagocytosis of $^{51}$Cr-labeled sheep RBC (as % of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of AMs</td>
<td>No. of PEC</td>
</tr>
<tr>
<td>(x 10$^6$)</td>
<td>(x 10$^6$)</td>
</tr>
<tr>
<td>Untreated control</td>
<td>25.4</td>
</tr>
<tr>
<td>Silica</td>
<td>14.5</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>17.3</td>
</tr>
<tr>
<td>Hyperchlorinated water</td>
<td>13.9</td>
</tr>
</tbody>
</table>

* Treatment protocols are defined in "Materials and Methods."

$^b$ Statistically significant from controls ($p < 0.002$).

$^c$ Statistically significant from controls ($p < 0.01$).

Table 3

<table>
<thead>
<tr>
<th>Pretreatment of mice with antimacrophage agent$^a$</th>
<th>Liposome treatment$^b, c$</th>
<th>No. of mice with metastasis/total</th>
<th>Median no. of pulmonary metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>None</td>
<td>12/15</td>
<td>39 (0–102)$^c$</td>
</tr>
<tr>
<td>No treatment</td>
<td>PC:PS (lymphokines)</td>
<td>34</td>
<td>9/14</td>
</tr>
<tr>
<td>No treatment</td>
<td>PC:PS (MDP)</td>
<td>48</td>
<td>4/14</td>
</tr>
<tr>
<td>Silica</td>
<td>PC:PS (lymphokines)</td>
<td>14$^b$</td>
<td>12/14</td>
</tr>
<tr>
<td>Silica</td>
<td>PC:PS (HBSS)</td>
<td>4$^b$</td>
<td>13/14</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>PC:PS (lymphokines)</td>
<td>3$^b$</td>
<td>12/14</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>PC:PS (HBSS)</td>
<td>9</td>
<td>12/14</td>
</tr>
<tr>
<td>Hyperchlorinated water</td>
<td>PC:PS (MDP)</td>
<td>21$^b$</td>
<td>9/12</td>
</tr>
<tr>
<td>Hyperchlorinated water</td>
<td>PC:PS (HBSS)</td>
<td>2$^b$</td>
<td>11/14</td>
</tr>
</tbody>
</table>

$^a$ Groups of mice were treated with the indicated agents prior to liposome therapy as described in "Materials and Methods."

$^b$ For the assays of in vitro-mediated cytotoxicity by AMs, control and test groups were given injections of PC:PS (7:3 mol ratio) multilamellar vesicle liposomes containing the indicated encapsulated materials as a single i.v. injection (5 µmol lipid per mouse) in 0.2 ml HBSS. Additional groups of control animals given injections of 0.2 ml HBSS alone or liposomes containing encapsulated HBSS suspended in HBSS containing free lymphokines (12.5 µg) or MDP (2.5 µg) in a total volume of 0.2 ml HBSS did not differ significantly from control animals given injections of liposome-encapsulated HBSS (data not shown).

$^c$ For treatment of spontaneous metastases arising from B16-BL6 cells implanted s.c., mice were given i.v. injections of the indicated liposomes containing HBSS, lymphokines, or MDP twice weekly for weeks.

$^d$ AMs were harvested from animals 24 hr after injection with liposomes and were assayed for their ability to destroy [125I]iododeoxyuridine-labeled B16-BL6 melanoma cells, using the 3-day cytotoxicity assay described in Table 1. Footnote b. The results represent mean values from 2 separate experiments. Deviations from the mean did not exceed 15%.

$^e$ Numbers in parentheses, range.

$^f$ Statistically significant reduction in metastasis as compared to untreated controls ($p < 0.001$).

$^g$ Statistically significant reduction in cytotoxicity compared to corresponding controls ($p < 0.001$).

$^h$ Statistically significant increase in metastatic burden compared to untreated control animals ($p < 0.01$).
lipo- somes containing macrophage activators is mediated by tumoricidal macrophages. (a) In animals given injections of liposomes that are not retained in the lung (PC), no activation of AMs or retardation of metastasis was observed. PC liposomes are not arrested efficiently in the lung vasculature and do not lead to the generation of tumoricidal properties of AMs or retardation of metastasis was observed. PC liposomes containing macrophage activators ineffective. Although the agents used to impair macrophage function are selective for macrophages (1-3, 5, 6, 25, 26, 29, 42, 44), the selectivity is not absolute. Both silica and carrageenan have been reported to alter functions mediated by lymphocytes (29, 32, 33) and natural killer cells (27, 31, 38). There is evidence to suggest, however, that the effects of silica and carrageenan observed in this study result primarily from their action on macrophages rather than on T-lymphocytes or natural killer cells. (a) Mice depleted of natural killer cells and T-lymphocytes resemble normal mice in that they show increased antitumor resistance after treatment with liposome-encapsulated lymphokines or MDP (data not shown). (b) The systemic activation of macrophages to the tumoricidal state by liposome-entrapped lymphokines or MDP appears to be independent of the thymus, since it can be accomplished in adult thymectomized and X-irradiated mice as well as in athymic nude mice (14). These types of data suggest that macrophages are the essential effector cell in the destruction of metastases. The data do not rule out the possibility that macrophages function as helper cells or that they recruit other host effector cells or factors to destroy tumors. More direct evidence to indicate that macrophages activated by liposome-encapsulated lymphokines or MDP can act directly as cytotoxic effector cells comes from the third set of experiments, the adoptive transfer studies. In these experiments, mice bearing experimental metastases were not treated with injections of liposomes containing macrophage activators; rather, mice were given i.v. injections of liposomes that had phagocytosed liposomes containing either MDP (activated) or HBSS (nonactivated). Macrophages rendered tumoricidal by incubation in vitro with liposome-encapsulated MDP produced a significant reduction in the metastatic burden when injected i.v. into tumor-bearing animals. This is consistent with previous reports in which adoptive transfer of macrophages activated in vitro reduced tumor growth in syngeneic mice (9, 11, 30).

The demonstration that the augmented antitumor response produced by liposome-encapsulated lymphokines or MDP is mediated by activated macrophages may be important in the development of cancer therapy modalities. There is a growing body of evidence that activated macrophages are major effector cells in host resistance to tumors (for review, see Refs. 10 and 15). Recent data also suggest that the progressive growth of neoplasms may be caused, in part, by immune deficiencies that frustrate macrophage involvement in tumor rejection (3, 15). One such example is the decreased ability of lymphocytes in animals bearing large tumors to interact with tumor cells and to release lymphokines that recruit and activate macrophages (40). If such a defect is common in the tumor-bearing host, administration of agents that attempt to augment antitumor responses by stimulating lymphokine production in vivo may prove to be of little value because of a preexisting functional lesion in ’target’ lymphocytes. In contrast, agents that act directly on macrophages would not be expected to encounter this problem. However, based on recent findings showing that the tumoricidal phenotype in activated macrophages persists for only a few days (35, 39) before macrophages become refractory to ’reactivation’ by physiological mediators such as lymphokines (35), a successful therapeutic agent will have to overcome this refractory state. In this respect, it should be noted that phagocytic uptake of lymphokines encapsulated in liposomes can successfully activate previously activated macrophages that are unresponsive to free lymphokines (35, 36).

The disappointing results obtained to date in clinical immuno-therapy trials using agents designed to stimulate specific immune responses against tumors have prompted renewed interest in nonspecific mechanisms of ’natural’ resistance to tumor mediated by macrophages and natural killer cells (21). We believe that liposomes offer particular promise for future therapeutic efforts to modify macrophage function. Liposomes injected i.v. are similar to other circulating particulate material in that they are taken up almost exclusively by RE cells in the liver and spleen and by circulating monocytes (for review, see Ref. 20). Therapeutic agents encapsulated within liposomes can thus be ’targeted,’ albeit passively, to macrophages. This highly selective delivery to macrophages should allow a reduction in the amount of drug needed to activate macrophages, thereby reducing the likelihood of toxicity problems arising from action on cells other than macrophages.

REFERENCES


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