Cancer Immunol Immunother (1986) 21: 169–173

Cancer Immunology Immunotherapy

Optimization and limitations of systemic treatment of murine melanoma metastases with liposomes containing muramyl tripeptide phosphatidylethanolamine

Isaiah J. Fidler

Department of Cell Biology (173), The University of Texas, M. D. Anderson Hospital and Tumor Institute at Houston, 6723 Bertner Avenue, Houston, Texas 77030, USA

Summary. The purpose of these studies was to determine the optimal conditions and limitations for the eradication of spontaneous melanoma metastases by the systemic administration of liposomes containing MTP-PE. Mice whose primary melanoma had been excised were given i.v. injections of liposomes at various schedules. Optimal treatment was achieved by twice weekly administration for 4 weeks (eight i.v. injections). Bioassays failed to reveal the presence of melanoma cells in lungs of mice surviving to day 250 of the experiment. The success of liposome treatment of metastases diminished when the first i. v. injection of liposomes-MTP-PE commenced on day 10 after surgical excision of the local melanoma, as compared with day 3 or day 7 after surgery. We conclude that the major limitation for macrophage-mediated destruction of metastases is the number of tumor cells in the lesions. Because of this limitation, it is unlikely that the systemic activation of macrophages could be used as a single modality for treatment of advanced metastases.

Introduction

The use of liposomes to deliver a variety of therapeutic agents to phagocytic cells in vivo has attracted considerable attention [1, 24, 27, 28]. To a certain extent, the distribution of systemically administered liposomes can be influenced by their size, phospholipid composition, and surface charge [28, 30, 32]. However, most circulating liposomes bind to and are endocytosed by cells belonging to the reticuloendothelial system, including peripheral blood monocytes. We and others have exploited this natural fate of liposomes to "target" (albeit passively) immunodulators such as lymphokines or muramyl peptides to macrophages in vivo [28, 32].

Lymphokines such as gamma interferon or macrophage-activating factor, muramyl dipeptide, or lipophilic derivatives of muramyl dipeptide, e.g., muramyl tripeptide phosphatidylethanolamine (MTP-PE), entrapped in liposomes can activate human or mouse macrophages to the tumoricidal state both in vitro [2, 5, 6, 7, 12, 14, 18–21, 26, 29, 31, 33, 34, 39] and in vivo [3, 4, 6–12, 17, 22–25, 26, 35, 36]. Moreover, we have reported that the repeated i. v. injection of liposomes containing immunomodulators such as MTP-PE into mice with established lung and lymph node melanoma metastases results in the eradication of the metastases, and the survival of at least 70% of the mice [6, 7, 11]. Subsequent studies have provided indirect [11] and direct [17] evidence that activated macrophages were indeed responsible for the destruction of the metastases in the liposome-treated mice.

Even under controlled laboratory conditions, 30% of the mice treated with liposomes containing MTP-PE died of extensive disease [6, 11]. These fatal tumor cells, however, were not resistant to macrophage-mediated lysis [6, 13]. Several questions thus remain unanswered: (1) could manipulation of the schedule of liposome-MTP-PE administration influence the outcome of the treatment? (2) What is the major factor distinguishing between the "treatment success" and "treatment failure" mice? (3) Were the surviving mice free of dormant metastatic cells in the lung? The present report concerns studies designed to answer these questions and the investigations of optimizing the systemic administration of liposome-MTP-PE for the treatment of spontaneous mouse melanoma metastases.

Materials and methods

Animals. Specific-pathogen-free C57BL/6N mice were obtained from the NCI-Frederick Cancer Research Facilities Animal Production Area. Mice used in each experiment were matched by age (6–8 weeks) and sex.

Tumor culture. B16-BL6, a tumor variant obtained by in vitro selection for invasion [16], originated from the spontaneous malignant B16 melanoma, which is syngeneic to the C57BL/6N mouse. The B16-BL6 tumor cells were grown as monolayer cultures and were maintained in Eagle's minimum essential medium supplemented with 5% fetal bovine serum, vitamin solution, sodium pyruvate, L-glutamine, and nonessential amino acids. The supplements and medium were obtained from M. A. Bioproducts, Walkersville, Md. The medium was endotoxin free as determined by the *Limulus* amoebocyte lysate assay (Associates of Cape Cod, Woods Hole, Mass.). Cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 in air. All cell cultures were free of mycoplasma, reovirus type 3, pneumonia virus of mice, K-virus, Theiler's encephalitis virus, lymphocytic choriomeningitis virus, ectromelia virus, and lactate dehydrogenase virus (assayed by M. A. Bioproducts, Walkersville, Md).

Offprint requests to: I. J. Fidler

Reagents. D-isoglutamyl-L-alanyl-2-(1',2'-dipalmitoyl-sn-glycero-3'-phosphoryl)-ethylamide (MTP-PE) was the gift of Ciba-Geigy Ltd., Basel, Switzerland. No endotoxins were detected in the MTP-PE by the *Limulus* amoebocyte lysate assay (Associates of Cape Cod).

Lipids and liposome preparation. Chromatographically pure egg phosphatidylcholine (PC) and beef brain phosphatidylserine (PS) were purchased from Avanti Polar Lipids, Inc., Birmingham, Ala. Multilamellar vesicles (MLV) were prepared as described previously [30] and used immediately. Briefly, PC, PS, and MTP-PE were dissolved in chloroform and then dried together by rotary evaporation and further dried in vacuo. The lipids were then hydrated in Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution (HBSS) and mechanically agitated on a vortex machine at room temperature. Control liposome preparations contained HBSS in their aqueous interior. The extent of incorporation of MTP-PE into the liposome preparation was assessed by employing trace amounts of ^{3}HMTP-PE admixed with MTP-PE [31]. In addition, the incorporation and proper orientation of MTP-PE into the phospholipid bilayer membrane was assessed using anti-MDP antibodies [10, 31].

Spontaneous metastasis system. To produce spontaneous lymph node and lung metastases, mice were inoculated in a hind footpad with 5×10^4 viable B16-BL6 cells suspended in a total volume of 0.05 ml HBSS. Then 4 to 5 weeks later, when the tumors had reached a diameter of 12–15 mm, the mice were anesthetized, and the tumor-bearing leg, including the popliteal lymph node, was amputated at the midfemur.

Schedule of liposome treatment. In this tumor system, spontaneous lung and lymph node metastases are well established by the time the primary tumor (in the foot pad) grows to 12–15 mm [3]. Systemic treatment of mice was usually initiated 3 days after removal of the primary tumor.

In all our previously published studies on the treatment of established metastasis by i. v. injected liposomes, the liposomes were injected twice per week for 4 weeks, a total of eight i. v. injections [3, 9]. In the present study we determined the influence of varying this schedule on the outcome of the treatment. Groups of mice ($n \geqslant 10$) whose primary B16-BL6 melanoma was resected 3 days previously received i. v. injections of liposomes containing MTP-PE according to the following schedules: group-1: once a week for 8 weeks; group-2: twice weekly for 1 week; group-3: twice weekly for 2 weeks; group-4: twice weekly for 3 weeks; and group-5: twice weekly for 4 weeks. Group-6 consisted of control mice injected twice weekly for 4 weeks with control liposome preparation containing HBSS.

All animals were monitored daily for 250 days. Dead or moribund animals were necropsied to ascertain the presence or absence of melanoma metastases. Animals surviving at least 100 days after the last liposome treatment were considered to be free of disease [9].

The influence of initial tumor burden on the outcome of liposome-MTP-PE treatment. In this tumor system by the time of surgicial resection of the local melanoma tumor (hind leg) metastases are well established. The tumor burden in the lung and lymph node metastases on day 3 after leg amputation has been estimated to be between 5×10^6 and 1×10^7 cells [3, 9]. The volume doubling time of B16-BL6 melanoma lung metastases ranges between 3 and 5 days [3, 15, 16]. For this reason we wished to determine whether the timing of the first liposome administration was critical to the outcome of the treatment. In the next set of experiments, the first i. v. injection of MLV-MTP-PE was administered to groups of mice ($n=20$) either 3 days, 7 days, or 10 days after the amputation of the hind footpad with the primary implanted melanoma. Control groups of mice were injected i. v. with a placebo preparation of liposomes (MLV-HBSS). All mice were treated twice weekly with liposome-MTP-PE or liposome-control for 4 weeks, for a total of eight i. v. injections. The mice were monitored daily for 250 days. Moribund or dead mice were necropsied to determine the presence or absence of melanoma metastases.

Bioassay for tumor cells in lung tissue. In the final set of experiments we examined whether the treatment success mice were harboring any metastatic cells in a "dormant" state. Since the B16-BL6 melanoma demonstrates preferential growth to the lung we concentrated our efforts on this organ. Lung tissue (from normal untreated mice) or from tumor-free, treatment success mice as well as from pulmonary B16-BL6 metastases (from treatment failure mice) were removed en bloc and minced into fragments measuring approximately 1 mm^3. The organ or tumor fragments were digested by continuous stirring in trypsinization flasks at 37 °C, as previously described [17, 37]. The digestion medium consisted of HBSS containing 0.14% collagenase type I and 0.10% deoxyribonuclease type I (Sigma Chemical Co., St. Louis, Mo.). Three 30-min digestion cycles produced 65%–85% digestion of the lung fragments to single cell suspensions. The suspension of single lung cells was adjusted to contain 5×10^6 lung cells/ml HBSS. The suspension of single tumor cells was adjusted to contain a final concentration of 1×10^3 cells/ml. Normal (control) mice were injected s. c. with either 5×10^6 lung cells from normal mice or with 5×10^6 lung cells from NR mice admixed with 100 B16-BL6 cells (1 ml and 0.1 ml of the two suspensions). Recipient mice were also injected s. c. with 5×10^6 cells dissociated from the lungs of eight different treatment success mice ($n=3$). All mice were monitored for 60 days for the growth of subcutaneous melanoma.

Statistical analysis of treatment studies. The survival data were analyzed by the Kruskal-Wallis test or the Cox's test or both [38].

Results

The effect of liposome schedule of administration on the outcome of treatment of metastases

The data of this experiment are shown in Fig. 1. Systemic treatment with liposomes (each dose consisted of 2.5 μmol lipid containing 10 μg MTP-PE) began on day 35–40 of the experiment (i.e., after implantation of B16-BL6 cells into the hind footpad and 3 days after the surgical removal of the local tumor). By day 100 of the experiments, 90% of

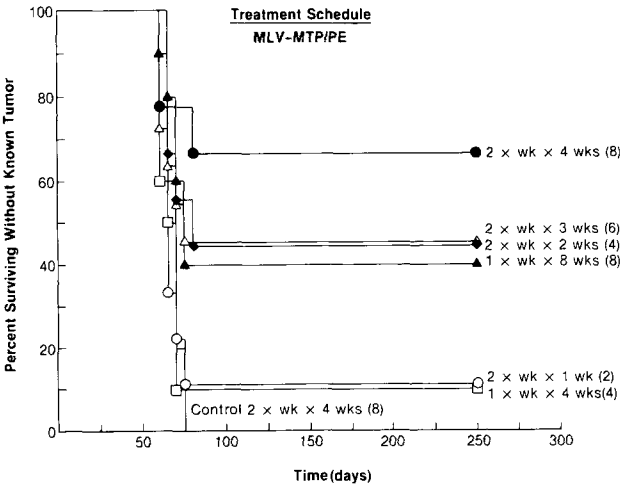

Fig. 1. Treatment of spontaneous lung and lymph node metastases by the systemic administration of MLV containing MTP-PE. Mice were injected i.v. with 2.5 μmol MLV containing 10 μg MTP-PE/dose. The schedule of liposome administration varied. Number in parentheses indicates the total number of i.v. treatments

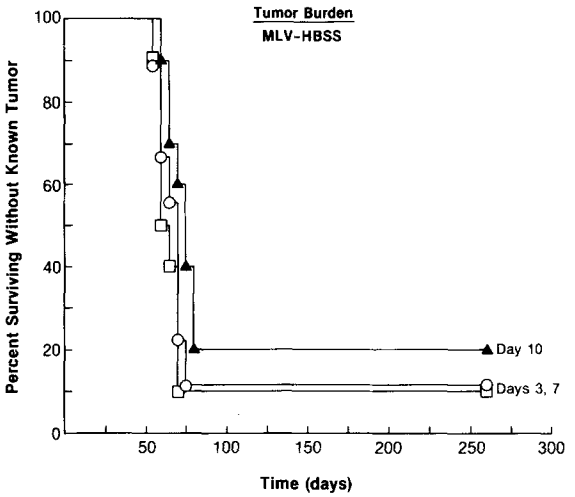

Fig. 2. Lack of effect on eradication of metastases by MLV containing HBSS. These are control mice for the groups shown in Fig. 1. Each i.v. injection (twice weekly for 4 weeks) consisted of 2.5 μmol phospholipids containing HBSS

the mice injected with control MLV (group-6) or mice in group-2 treated with MLV-MTP-PE twice per week for only 1 week had died. As can be readily seen, practically all mice surviving on day 100 of the experiment were alive on day 250 of the study when it was terminated. The most effective schedule for treatment of lung metastases was the twice weekly injection for 4 weeks (group-5): 68% of the mice were alive on day 250 of the experiment ($P<0.001$). Less impressive but still statistically significant survival was observed in the following groups: group-1 treated once weekly for 8 weeks (40% survival, $P<0.01$); group-3 treated twice weekly for 2 weeks (44% survival, $P<0.01$) and group-4 treated twice weekly for 3 weeks (45% survival, $P<0.001$).

The influence of initial tumor burden on the outcome of liposome-MTP-PE treatment

The data of these experiments are shown in Fig. 2 (liposomes containing HBSS) and Fig. 3 (liposomes containing MTP-PE). The systemic administration (eight i.v. injections) of liposomes containing HBSS had no therapeutic benefits regardless of the time of initial treatment (Fig. 2). Treatment with liposomes containing MTP-PE (Fig. 3) had significant therapeutic benefits, but the degree of success depended upon the timing of the first treatment (of eight i.v. injections). The most impressive survival was observed in mice treated with MLV containing MTP-PE beginning on day 3 after surgical removal of the primary tumor (65% survival, 13/20 mice, $P<0.001$). If the first MLV-MTP-PE treatment began on day 7 after surgical excision of the primary tumor, 45% (9/20) of the mice survived ($P<0.01$). In the last group the first MLV-MTP-PE treatment began on day 10 after surgical excision of the primary tumor, and here only 33% (6/20) of the mice survived ($P=0.2$).

Bioassay for dormant tumor cells in lungs of mice

In the final set of experiments, we investigated whether the lungs of animals surviving at day 200–250 of the study contained dormant melanoma cells. Lung cells of mice

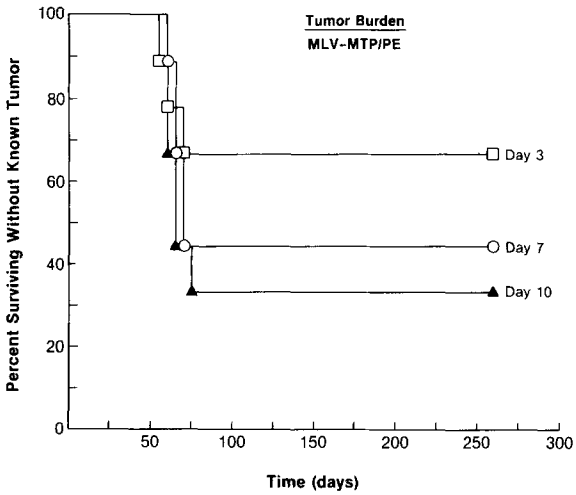

Fig. 3. The influence of initial tumor cell number in metastases on the outcome of treatment. Primary cutaneous melanomas were excised and mice received the first i.v. treatment of MLV-MTP-PE either on day 3, day 7, or day 10 after surgery. All mice received 8 i.v. injections (twice weekly for 4 weeks). Each treatment dose consisted of 2.5 μmol phospholipids containing 10 μg of MTP-PE

were dissociated, and 5×10^6 lung cells were injected s.c. into normal syngeneic recipients (3 mice per group). No subcutaneous tumors developed in the 24 mice injected with 5×10^6 cells dissociated from lungs of 8 tumor-free mice. In contrast, 5×10^6 lung cells from normal mice admixed with 100 viable B16 melanoma cells produced subcutaneous tumors in 19 of 20 injected mice. These data suggest, therefore, that the 5×10^6 lung cells of tumor-free mice contained less than 100 viable B16 cells.

Discussion

In these experiments, particular attention was given to the optimal conditions required for successful eradication of lymph node and lung metastases by the systemic adminis-

tration of liposomes containing MTP-PE. The ability to activate alveolar macrophages in situ with MLV containing MTP-PE [4, 6, 9, 10, 12] is attractive for several reasons. First, virtually all of the compound used in the preparation of the MLV is incorporated into the liposome structure, in contrast to the significant waste that occurs in the preparation of MLV containing hydrophilic MDP [10]. Second, because MTP-PE is firmly associated with the phospholipid bilayer of the MLV, it is efficiently delivered to macrophages and, once phagocytosed, results in consistent and reproducible activation of macrophages in situ [10]. Third, once prepared, MLV containing MTP-PE retain their ability to activate alveolar macrophages in situ for 8–12 weeks after storage at 4 °C [31].

The attributes of MLV containing MTP-PE may account for the results obtained in the therapy experiments. Of the mice treated twice weekly with MLV containing MTP-PE 68% were alive by day 250 of the experiment (170 days after the final treatment). This period exceeds the time necessary (40–50 days) for as few as 100 surviving tumor cells to kill their host [15]. Optimal success in eradicating metastases was obtained by the twice weekly administration of MLV-MTP-PE for 4 weeks (eight i.v. injections). Injecting the MLV once a week for 8 weeks (eight i.v. injections) was not as therapeutic. Thus, the optimal treatment directly correlated with the in vivo half-life of the MLV-MTP-PE and the duration of macrophage activation to a tumoricidal state [10, 12]. It is entirely conceivable that improvement in treatment could have occurred with a longer biweekly schedule (8 weeks or even 12 weeks). Technical difficulties of tail vein injections did not allow us to thoroughly study this possibility.

Even under optimal conditions, not all the treated mice survived. We have previously shown that those "treatment failure" mice did not die from the proliferation of tumor cells resistant to macrophage-mediated lysis [13]. In the present study, we found that the degree of success or failure in treatment of spontaneous metastases by i.v. injections of MLV-PE was directly related to the tumor burden at the time of initial treatment. This conclusion is based on the data of the studies illustrated in Figs. 2 and 3. In this B16 tumor system, the volume doubling time of metastases was 3–5 days [3, 15]. On day 3 after surgery of the primary melanoma, the number of tumor cells in metastases is estimated to be less than 10^7 cells. The number of metastatic cells obviously increases with time and could reach 3×10^7 cells by 10 days after resection of the local tumor. At this time, the lung metastases are large and can be seen without microscopy [7]. Indeed, the percentage of surviving mice decreased dramatically when the first i.v. MLV treatment began on day 10.

Mice surviving until day 250 of the experiment were probably free of tumor cells. This conclusion is based on the data in which appropriate bioassays of lungs from treatment success mice failed to reveal the presence of dormant melanoma cells.

In conclusion, the results shown here recommend that the schedule of administrating MLV containing MTP-PE that optimally activates tumoricidal properties in macrophages [4, 10, 12, 30, 31] is also optimal for the treatment of systemic metastases. Since the major limitation in the treatment of disseminated cancer with macrophages activated in situ is not tumor cell resistance to macrophage-mediated lysis [13] but rather the inability of macrophages to destroy a large number of tumor cells, the data presented here suggest that macrophage-mediated destruction of large tumor burdens may not be feasible. For this reason the potential application of macrophage activation in situ should not be directed towards the destruction of large tumors, but rather the destruction of micrometastases or the few tumor cells that resist conventional therapy of large lesions.

References

1. Alving CR (1983) Delivery of liposome-encapsulated drugs to macrophages. Pharmacol Ther 22: 407
2. Deodhar SD, Barna BP, Edinger M, Chiang T (1982) Inhibition of lung metastases by liposomal immunotherapy in a murine fibrosarcoma model. J Biol Response Mod 1: 27
3. Fidler IJ (1980) Therapy of spontaneous metastases by intravenous injection of liposomes containing lymphokines. Science 208: 1469
4. Fidler IJ (1981) The in situ induction of tumoricidal activity in alveolar macrophages by liposomes containing muramyl dipeptide is a thymus-independent process. J Immunol 127: 1719
5. Fidler IJ, Kleinerman ES (1984) Lymphokine-activated human blood monocytes destroy tumor cells but not normal cells under cocultivation conditions. J Clin Oncol 2 (8): 937
6. Fidler IJ, Poste G (1982) Macrophage-mediated destruction of malignant tumor cells and new strategies for the therapy of metastatic disease. Springer Semin Immunopathol 5: 161
7. Fidler IJ, Schroit AJ (1984) Synergism between lymphokines and muramyl dipeptide encapsulated in liposomes: In situ activation of macrophages and therapy of spontaneous cancer metastasis. J Immunol 133 (1): 515
8. Fidler IJ, Raz A, Fogler WE, Kirsh R, Bugelski P, Poste G (1980) The design of liposomes to improve delivery of macrophage-augmenting agents to alveolar macrophages. Cancer Res 40: 4460
9. Fidler IJ, Sone S, Fogler WE, Barnes ZL (1981) Eradication of spontaneous metastases and activation of alveolar macrophages by intravenous injections of liposomes containing muramyl dipeptide. Proc Natl Acad Sci USA 78: 1680
10. Fidler IJ, Sone S, Fogler WE, Smith D, Braun DG, Tarcsay L, Gisler RJ, Schroit AJ (1982) Efficacy of liposomes containing a lipophilic muramyl dipeptide derivative for activating the tumoricidal properties of alveolar macrophages in vivo. J Biol Response Mod 1: 43
11. Fidler IJ, Barnes Z, Fogler WE, Kirsh R, Bugelski P, Poste G (1982) Involvement of macrophages in the eradication of established metastases following intravenous injection of liposomes containing macrophage activators. Cancer Res 42: 496
12. Fidler IJ, Fogler WE, Tarcsay L, Schumann G, Braun DG, Schroit AJ (1983) Systemic activation of macrophages and treatment of cancer metastasis by liposomes containing hydrophilic or lipophilic muramyl dipeptide. In: Advances in immunopharmacology, vol. 2, Pergamon Press: Oxford, p 235
13. Fogler WE, Fidler IJ (1985) Nonselective destruction of murine neoplastic cells by syngeneic tumoricidal macrophages. Cancer Res 45: 14
14. Fogler WE, Raz A, Fidler IJ (1980) In situ activation of murine macrophages by liposomes containing lymphokines. Cell Immunol 53: 214
15. Griswold DP Jr (1972) Consideration of the subcutaneously implanted B16 melanoma as a screening model for potential anticancer agents. Cancer Chemother Rep 3: 315
16. Hart IR (1979) The selection and characterization of an invasive variant of the B16 melanoma. Am J Pathol 97: 587
17. Key ME, Talmadge JE, Fogler WE, Bucana C, Fidler IJ (1982) Isolation of tumoricidal macrophages from lung mela-

noma metastases of mice treated systemically with liposomes containing a lipophilic derivative of muramyl dipeptide. J Natl Cancer Inst 69: 1189
18. Kleinerman ES, Schroit AJ, Fogler WE, Fidler IJ (1983) Tumoricidal activity of human monocytes activated in vitro by free and liposome-encapsulated human lymphokines. J Clin Invest 72: 1
19. Kleinerman ES, Erickson KL, Schroit AJ, Fogler WE, Fidler IJ (1983) Activation of tumoricidal properties in human blood monocytes by liposomes containing lipophilic muramyl tripeptide. Cancer Res 43: 2010
20. Kleinerman ES, Zicht R, Sarin PS, Gallo RC, Fidler IJ (1984) Constitutive production and release of a lymphokine with macrophage-activating factor activity distinct from gamma-interferon by a human T-cell leukemia viruspositive cell line. Cancer Res 44: 4470
21. Lopez-Berestein G, Mehta K, Mehta R, Juliano RL, Hersh EM (1983) The activation of human monocytes by liposome-encapsulated muramyl dipeptide analogues. J Immunol 130: 1500
22. Lopez-Berestein G, Milas L, Hunter N, Mehta K, Eppstein D, VanderPas MA, Mathews TR, Hersh EM (1984) Prophylaxis and treatment of experimental lung metastases in mice after treatment with liposome encapsulated 6-O-steroyl-N-acetyl-muramyl-L-aminobutyryl-D-isoglutamine. Clin Exp Metastasis 2 (2): 336
23. Mehta K, Lopez-Berestein G, Hersh EM, Juliano RL (1982) Uptake of liposomes and liposome-encapsulated muramyl dipeptide by human peripheral blood monocytes. J Reticuloendothel Soc 32: 155
24. Nicolau C, Paraf A (eds), (1981) Liposomes, drugs and immunocompetent cell functions, Academic Press, London
25. Phillips NC, Mora ML, Chedid L, Lefrancier P, Bernard JM (1985) Activation of tumoricidal activity and eradication of experimental metastases by freeze-dried liposomes containing a new lipophilic muramyl dipeptide derivative. Cancer Res 45: 128
26. Poste G, Kirsh R, Fogler W, Fidler IJ (1979) Activation of tumoricidal properties in mouse macrophages by lymphokines encapsulated in liposomes. Cancer Res 39: 881
27. Poste G, Bucana C, Raz A, Bugelski P, Kirsh R, Fidler IJ (1982) Analysis of the fate of systemically administered liposomes and implications for their use in drug delivery. Cancer Res 42: 1412
28. Poste G, Kirsh R, Bugelski P (1984) Liposomes as a drug delivery system in cancer therapy. In: Sunkara P (ed) Novel approaches to cancer chemotherapy. Academic Press, New York, p 323
29. Saiki I, Fidler IJ (1985) Synergistic activation by recombinant mouse gamma-interferon and muramyl dipeptide of tumoricidal properties in mouse macrophages. J Immunol 135: 684
30. Schroit AJ, Fidler IJ (1982) Effects of liposome structure and lipid composition on the activation of the tumoricidal properties of macrophages by liposomes containing muramyl dipeptide. Cancer Res 42: 161
31. Schroit AJ, Galligioni E, Fidler IJ (1983) Factors influencing the in situ activation of macrophages by liposomes containing muramyl dipeptide. Biol Cell 47: 87
32. Schroit AJ, Hart IR, Madsen J, Fidler IJ (1983) Selective delivery of drugs encapsulated in liposomes: Natural targeting to macrophages involved in varius disease states. J Biol Response Mod 2: 97
33. Sone S, Fidler IJ (1980) Synergistic activation by lymphokines and muramyl dipeptide of tumoricidal properties in rat alveolar macrophages. J Immunol 125: 2454
34. Sone S, Fidler IJ (1981) In vitro activation of tumoricidal properties in rat alveolar macrophages by synthetic muramyl dipeptide encapsulated in liposomes. Cell Immunol 57: 42
35. Sone S, Tsubura E (1982) Human alveolar macrophages: Potentiation of their tumoricidal activity by liposome-encapsulated muramyl dipeptide. J Immunol 129: 1313
36. Sone S, Poste G, Fidler IJ (1980) Rat alveolar macrophages are susceptible to activation by free and liposome-encapsulated lymphokines. J Immunol 124: 2197
37. Talmadge JE, Key M, Fidler IJ (1981) Macrophage content of metastatic and nonmetastatic rodent neoplasms. J Immunol 126: 2245
38. Thomas DG, Breslow N, Gart JJ (1977) Trend and homogeneity analyses of proportions and life table data. Comput Biomed Res 10: 373
39. Xu ZL, Bucana CD, Fidler IJ (1984) In vitro activation of murine Kupffer cells by lymphokines or endotoxins to lyse syngeneic tumor cells. Am J Pathol 117: 372

Received June 14, 1985/Accepted October 2, 1985