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Potential Value of Cetylmannoside-modified Liposomes as Carriers of Macrophage Activators to Human Blood Monocytes

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The present study was undertaken to examine the potential value of cetylmannoside-modified multilamellar liposomes (Man-MLV) as carriers for transfer of macrophage activators to blood monocytes. Highly purified blood monocytes were isolated by centrifugal elutriation from healthy donors under endotoxin-free conditions. Freshly prepared monocytes phagocytosed Man-MLV to a lesser extent than monocyte-derived macrophages, but they took up Man-MLV much more effectively than control liposomes without cetylmannoside (control MLV). Phagocytosis of Man-MLV, but not control MLV by monocytes was inhibited by addition of D-mannose, but not of D-galactose. Desmethyl-muramyl dipeptide (norMDP) entrapped in Man-MLV was far more effective than norMDP entrapped in MLV in activating monocytes to the tumoricidal state. The effect of encapsulation of recombinant human macrophage colony-stimulating factor (M-CSF) in Man-MLV on prolongation of survival of monocytes was examined. Blood monocytes that had been incubated for up to 21 days with Man-MLV containing 5–20 U of M-CSF per ml were effective in prolonging monocyte survival, but monocytes that had been incubated in medium with less than 50 U/ml of M-CSF or with control MLV containing 5–10 U of M-CSF showed no increase of monocyte survival over that in medium alone. Addition of rabbit anti-M-CSF antiserum did not affect survival prolongation of monocytes by M-CSF encapsulated in Man-MLV. We conclude that liposomes modified with cetylmannoside are far more effective than unmodified liposomes as a carrier to deliver biological response modifiers to human blood monocytes.

Key words: Cetylmannoside — Liposome — Monocyte-macrophage — Cytotoxicity — Survival

There is increasing evidence that activated macrophages are important in host defense against primary and/or metastatic tumors in animal models. These findings have led to the discovery of new biological response modifiers (BRMs) that can augment macrophage-mediated tumor cell killing.[1,2] Human monocyte-macrophages were also found to be activated to the tumoricidal state by various activation stimuli such as bacterial preparations and bioproducts.[3–5]

Recently, much attention has been paid to the use of multilamellar vesicles (MLV) as carriers of BRMs to macrophage-histiocytes. Various BRMs encapsulated in MLV were found to activate murine macrophages *in vitro* and *in vivo*.[1,2,6–8] Multiple injections of BRMs in liposomes also eradicated spontaneous pulmonary and lymph node melanoma metastases in mice.[1,2,6–8]

Mature macrophages, such as alveolar,[9,10] peritoneal[11] and bone marrow-derived macrophages[12] have been found to possess mannose-specific receptors, which mediate endocytosis of specific macromolecular ligands. In fact, mannosylated liposomes have been shown to be effective carriers of drugs targeted to macrophages.[13–16] In contrast, freshly isolated human monocytes were found not to express mannose receptors.[17] Nevertheless, it is important to know whether mannose-binding sites are present on freshly isolated blood monocytes, because circulating monocytes are the first cells that intravenously injected MLV encounter. Roche *et al.*[18] demonstrated by quantitative flow cytofluorometry that freshly isolated human monocytes bind mannose 6-phosphate and actively endocytose mannose 6-phosphate-bearing neoglycoproteins, indicating that these cells do express mannose-specific membrane lectins. This suggests that these lectins may play a role as adhesion molecules for Man-MLV to be phagocytosed by fresh monocytes. In the present study, we found that freshly isolated human blood monocytes phagocytosed Man-MLV preferentially over unmodified MLV, and that Man-MLV were more effective than unmodified MLV as a carrier to deliver BRMs (norMDP and M-CSF) to blood monocytes *in vitro*.

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MATERIALS AND METHODS

Reagents Fetal bovine serum (FBS) was purchased from M. A. Bioproducts (Walkersville, MD). Recombinant human M-CSF (specific activity, 1×10^7 U/mg protein) and rabbit antiserum raised against human recombinant M-CSF were gifts from Otsuka Pharmaceutical Co. (Tokushima). norMDP was a gift from Ciba-Geigy (Basel). Hydrogenated egg phosphatidylcholine (PC) was kindly supplied by Nippon Fine Chemicals Co. (Osaka). Dicetyl phosphate (DCP) was purchased from Nacalai Tesque (Kyoto). Cholesterol (CH), D-mannose, D-galactose and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) were purchased from Wako Pure Chemicals Industries (Osaka). Cetylmannoside (Man) was synthesized as described previously.[19] All these reagents were free of endotoxins as determined by *Limulus* amebocyte lysate assay (sensitivity limit, 0.1 ng/ml). LPS (*E. coli* 055:B5) was obtained from Difco Laboratories (Detroit, MI).

Cell line A375 cells derived from a human melanoma were adapted to growth in culture.[3,4] All cultures were free of mycoplasma and were maintained on plastic in RPMI 1640 supplemented with 10% heat-inactivated FBS and gentacin, designated as CRPMI 1640, at 37°C in a humidified atmosphere of 5% CO_2 in air. Cytotoxicity assays were performed when the cultured target cells were in the exponential phase.

Preparation of multilamellar liposomes (MLV) MLV were prepared as described previously.[2,19] Briefly, appropriate lipids were mixed in chloroform and dried under reduced pressure. The lipids (50 μmol of total lipids) were then hydrated in 1 ml of RPMI 1640 containing recombinant human M-CSF (10,000 U/ml) or norMDP (20 μg/ml), and mechanically vortexed at room temperature. Unencapsulated materials were removed by washing the MLV by centrifugation at 3,000 rpm for 15 min. Control MLV were composed of PC/DCP/CH in a molar ratio of 5:1:4. Man-MLV were composed of PC/Man/DCP/CH in a molar ratio of 2:3:1:4. The volumes of the aqueous interior of control MLV and Man-MLV were determined by measuring incorporation of carboxyfluorescein. The internal volumes of control MLV and Man-MLV expressed in μl/μmol were 10.3 and 10.2, respectively.

Isolation and culture of human blood monocytes Leukocyte concentrates were collected from peripheral blood (200 ml) in a Kubota KR-400 centrifuge with an RS-6600 rotor, and mononuclear cells were separated from the leukocyte concentrates in lymphocyte separation medium (LSM). Then, monocytes were isolated from the mononuclear cell samples by centrifugal elutriation in a Hitachi SRR6Y elutriation rotor.[3,4] A fraction containing more then 95% of the total monocyte population was obtained at 2,000 rpm and a flow rate of 20 ml/min. More than 90% of these cells were monocytes as determined by non-specific esterase staining and morphological examination, and more than 97% were viable, as judged by the Trypan-blue dye exclusion test. This fraction was washed twice with RPMI 1640 medium, and resuspended in CRPMI 1640 supplemented with 5% FBS, at a concentration of 1×10^6 monocytes per ml. These cells were then incubated for 2 h in 96-well Microtest III plates (Falcon, Oxnard, CA). Then, non-adherent cells were removed by washing with the medium. The purity of the remaining monocytes was >99% as judged from their morphology and the result of non-specific esterase staining.

***In vitro* treatment of monocytes** Monocyte monolayers were incubated for 24 h with or without norMDP entrapped in control MLV and Man-MLV before assay of monocyte-mediated cytotoxicity.

Monocyte-mediated cytotoxicity Cytotoxicity was assayed by measuring release of radioactivity as described in detail previously.[3,4] Target cells in the exponential growth phase were incubated for 24 h in CRPMI 1640 with 0.4 μCi/ml of [^{125}I]iododeoxyuridine (specific activity, 5 Ci/mg: Amersham International, Little Chalfont, UK). Inocula of 1×10^4 target A375 cells were plated into wells containing 1×10^5 monocytes, and 16 h later were washed to remove non-adherent and dead cells, and refed with fresh CRPMI 1640. After further incubation for 56 h, the monocyte/target cell cultures were washed twice with phosphate-buffered saline (PBS). Then adherent (presumably viable) cells were lysed with 0.1 ml of 0.1 *N* NaOH, and their radioactivity was measured in a gamma counter. The percent cytotoxicity mediated by activated monocytes was calculated as follows:

$$\%\ \text{Cytotoxicity} = \frac{A-B}{A}\times 100$$

where A represents cpm in the culture of untreated monocytes and target cells, and B represents cpm in the culture of test monocytes and target cells.

MTT assay Monocytes were incubated in suspension in CRPMI 1640 for the indicated times with or without free M-CSF or M-CSF entrapped in control MLV or Man-MLV, and then viable cells were measured by MTT assay.[20] Briefly, MTT was dissolved in PBS at 0.5 mg/ml and filtered for sterilization and removal of a small amount of insoluble residue present in some batches of MTT. At the times indicated below, stock MTT solution (50 μl per 100 μl medium) was added to all wells of the assay, and the plates were incubated at 37°C for 16 h. Then 10% sodium dodecyl sulfate in 0.01 *N* HCl was added to all wells and mixed thoroughly to dissolve the dark blue crystals. The plates were allowed to stand for 4 h at room temperature to ensure that all the crystals were

dissolved, and then examined with an MTP-32 Microtiter counter (Corona Electric, Ibaragi), using a test wavelength of 550 nm and a reference wavelength of 630 nm.

Assay of phagocytosis Monocytes or monocyte-derived macrophages were incubated in suspension with control MLV or Man-MLV containing 3 mol% of N-(lissamine rhodamine-B-sulfonyl) diacylphosphatidylethanolamine (N-Rh-PE; Avanti Polar Lipids, Inc., Birmingham, AL) for 3 h at 37°C. The cells were then washed with PBS, and their fluorescence intensity was measured in a FACScan (Becton Dickinson, Mountain View, CA) equipped with an argon ion laser operated at 488 nm and at 15 mW light output. Laser excitation was at 585 nm. After exclusion of dead cells and non-cell-associated liposomes by light-scattering measurements, the fluorescence intensity of 10,000 cells in each sample was analyzed. Acquired data were analyzed with a Consort 30 (Becton Dickinson).

Statistical analysis The statistical significance of differences between test groups was analyzed by using Student's *t* test.

RESULTS

Enhancing effect of modification of liposomes with cetylmannoside on their phagocytosis by blood monocytes The phagocytosis of control MLV and Man-MLV containing fluorescent N-Rh-PE was examined. For this, human blood monocytes were incubated for 3 h in medium with various concentrations of control MLV or Man-MLV containing fluorescent N-Rh-PE. The phagocytosis of Man-MLV by monocytes was significantly greater than that of control MLV at all concentrations examined (Fig. 1).

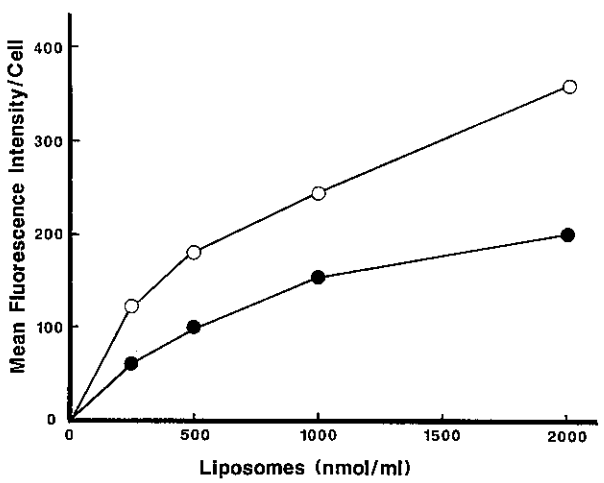

Fig. 1. Enhancing effect of cetylmannoside modification on uptake of MLV by monocytes. Monocytes were incubated for 3 h in medium with or without various concentrations of control MLV(●) or Man-MLV (○) containing N-Rh-PE. Data are representative of three separate experiments with essentially the same results. Points are means for 10,000 monocytes.

Blood monocytes possess lectin-like sites that can bind mannose.[18,21] Therefore, we examined whether phagocytosis of Man-MLV by blood monocytes was due to their binding of mannose on Man-MLV. For this, monocytes were incubated in medium containing 500 nmol/ml of liposome-N-Rh-PE with or without 50 m*M* D-mannose or D-galactose for 3 h. Then the cultures were washed and the fluorescence intensities associated with the monocytes were measured as described in "Materials and Methods." As shown in Table I, addition of D-mannose inhibited about 32% of the uptake of Man-MLV by fresh blood monocytes, but had little effect on phagocytosis of control MLV by monocytes. Under the same experimental conditions, D-galactose had no effect on phagocytosis of control MLV or Man-MLV by monocytes.

***In vitro* activation of human monocyte tumoricidal activity by norMDP encapsulated in Man-MLV** Blood monocytes were incubated for 24 h in endotoxin-free medium with or without free norMDP or norMDP encapsulated in Man-MLV at various concentrations of lipids and then their cytotoxicities against A375 human melanoma cells were assayed. The results are shown in Fig. 2. Human blood monocytes were rendered tumoricidal by *in vitro* treatment with much lower concentrations of norMDP in Man-MLV than of free norMDP. Control MLV containing norMDP without cetylmannoside modification also activated blood monocytes to the tumoricidal state, but were less effective than Man-MLV containing norMDP.

Table I. Uptake of Man-MLV by Blood Monocytes and Its Inhibition by D-Mannose

No. of exp.	Treatment[a]	Fluorescence intensity[b]	
		Control MLV	Man-MLV
1	Medium	229.7	411.6
	D-Mannose	208.3 (9%)[c]	279.5 (32%)
	D-Galactose	210.9 (8%)	381.4 (7%)
2	Medium	160.9	343.3
	D-Mannose	149.7 (7%)	238.1 (31%)
	D-Galactose	155.9 (3%)	321.1 (7%)

a) Blood monocytes were incubated for 3 h in medium with 500 nmol/ml of MLV or Man-MLV containing N-Rh-PE in the presence or absence of 50 m*M* D-mannose or D-galactose. Fluorescence intensity was measured as described in "Materials and Methods."

b) Means for 10,000 monocytes.

c) Values in parentheses show % inhibition as compared with that by monocytes incubated in medium alone.

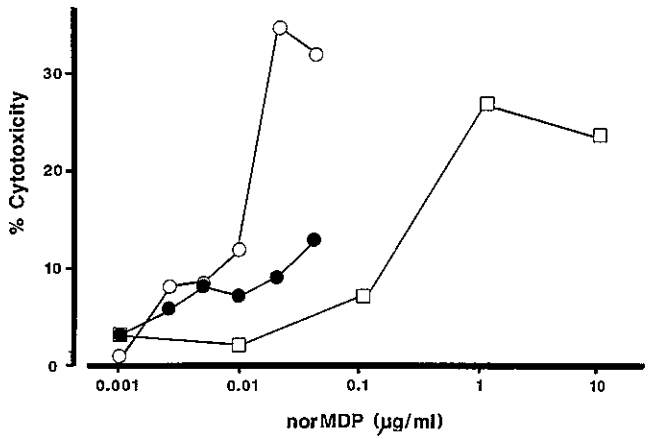

Fig. 2. *In vitro* tumoricidal activity of human monocytes treated with control MLV or Man-MLV at different concentrations of lipids. norMDP (20 μg/ml) diluted with RPMI 1640 was entrapped in control MLV or Man-MLV. Free norMDP (□), control MLV- (●) or Man-MLV (○) encapsulated norMDP at various concentrations of lipids was incubated with 1×10^5 monocytes for 24 h. Then the cells were washed and their cytotoxicity towards labeled A375 melanoma cells was assayed at an E/T ratio of 10:1. Incubations were terminated 72 h later. Cytotoxicity towards tumor cells was determined as a percentage of that of untreated monocytes.

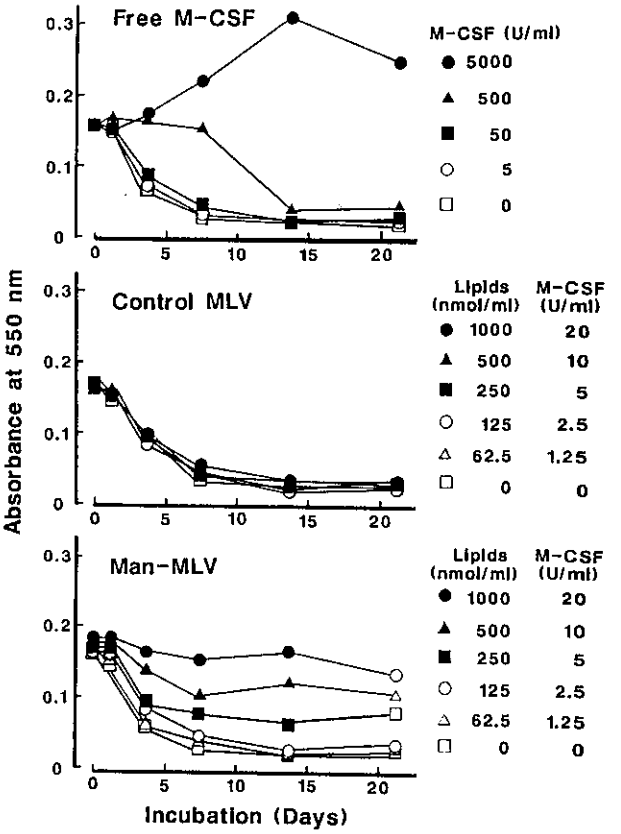

Fig. 3. Prolongation of survival of human blood monocytes by recombinant M-CSF in Man-MLV. Blood monocytes were incubated for up to 21 days in medium with or without free M-CSF at concentrations of 5 U/ml to 5,000 U/ml, control MLV or Man-MLV containing M-CSF at various concentrations of lipids in the range of 62.5 to 1,000 nmol/ml. At the indicated times, the cell numbers were counted by MTT assay as described in "Materials and Methods."

Prolongation of survival of blood monocytes by treatment with M-CSF in Man-MLV Blood monocytes require M-CSF for survival in medium with or without FBS.[22] Therefore, we compared the effects of free M-CSF, and M-CSF encapsulated in control MLV and Man-MLV on survival of blood monocytes. In this experiment, recombinant human M-CSF (100 U/μmol of total lipids) was encapsulated in control MLV and Man-MLV as described in "Materials and Methods," and blood monocytes were incubated for various times up to 21 days in medium with various concentrations of free M-CSF or M-CSF encapsulated in control MLV or Man-MLV. At the indicated times, the survival of the cells was determined by MTT assay. The experiment was repeated 5 times with similar results and representative data are shown in Fig. 3. Blood monocytes treated with more than 500 U/ml of free M-CSF survived significantly longer than those in medium alone or in medium with less than 50 U/ml of M-CSF. In contrast, control MLV with or without M-CSF did not affect monocyte survival at all. Under the same experimental conditions, treatment of monocytes with more than 250 nmol of M-CSF in Man-MLV resulted in significant, dose-dependent prolongation of monocyte survival. Prolongation of monocyte survival required at least 500 U/ml of free M-CSF, but only 10 U of M-CSF entrapped in Man-MLV per ml in culture medium. Thus, M-CSF encapsulated in Man-MLV was far more effective than free M-CSF or M-CSF in control MLV for prolonging monocyte survival.

In a parallel experiment, we examined whether Man-MLV prolonged monocyte survival by low concentrations of free M-CSF incapable of supporting the survival. Monocytes were incubated for 7 days in medium with or without free M-CSF and/or empty Man-MLV. As a positive control monocytes were treated with medium containing M-CSF encapsulated in Man-MLV. As shown in Table II, addition of M-CSF (10 U/ml) to cultures of monocytes with empty Man-MLV did not prolong monocyte survival as compared with that of cells treated with M-CSF in Man-MLV.

Effect of treatment with anti-M-CSF antiserum on prolongation of survival of monocytes by M-CSF in Man-MLV To rule out the possibility that M-CSF that leaked

Table II. Absence of Effect of Empty MLV with or without Cetylmannoside on Prolongation of Monocyte Survival

Treatment[a]	Absorbance at 550 nm[b]	
	Medium	M-CSF (10 U/ml)
Medium	0.033±0.015	0.030±0.005
Control MLV (empty)	0.029±0.002	0.034±0.005
Man-MLV (empty)	0.033±0.005	0.046±0.008
Control MLV (M-CSF 10 U/ml)	0.044±0.004	0.048±0.013
Man-MLV (M-CSF 10 U/ml)	0.104±0.005[c]	0.133±0.012[d]

a) Blood monocytes were incubated for 7 days in medium with or without the indicated agents in the presence or absence of free M-CSF (10 U/ml), and then monocyte survival was measured as described in "Materials and Methods."
b) Mean±SD for triplicate cultures. Data are representative of three separate experiments.
c) Significantly different from the value for monocytes incubated in medium alone ($P<0.05$).
d) Significantly different from the value for monocytes incubated in 10 U/ml of M-CSF alone ($P<0.05$).

Table III. Effect of Treatment with Anti-M-CSF Antiserum on Monocyte Survival in the Presence of M-CSF Entrapped in Man-MLV

Treatment[a]	Absorbance at 550 nm[b]	
	Medium	Anti-M-CSF antiserum
Medium	0.034±0.006	0.030±0.005
Free M-CSF (500 U/ml)	0.189±0.008	0.033±0.001
Man-MLV (M-CSF 5 U/ml)	0.168±0.017	0.173±0.007

a) M-CSF alone or M-CSF entrapped in Man-MLV was preincubated for 3 h in the presence or absence of anti-M-CSF antiserum. Then the mixtures were added to cultures of monocytes (1×10^5 cells/well). After 7 days, monocyte survival was measured by MTT assay.
b) Mean±SD for triplicate cultures. Data are representative of three separate experiments.

from Man-MLV or that was bound to the outer layers of the MLV mediated monocyte survival, we examined whether anti-M-CSF antiserum affected the prolongation of monocyte survival by M-CSF entrapped in Man-MLV. For this, 500 U/ml of free M-CSF or 250 nmol of M-CSF encapsulated in Man-MLV was incubated for 16 h in medium with anti-M-CSF antiserum, and then the mixtures were added to monocyte monolayers. After incubation for 7 days, the survival of the cells was measured by MTT assay. Representative results of three separate experiments are shown in Table III. Anti-M-CSF antiserum at 40-fold dilution almost completely neutralized 500 U/ml of M-CSF activity, but this concentration of anti-M-CSF antiserum did not affect the prolongation of monocyte survival by M-CSF encapsulated in Man-MLV.

Prolongation of survival of monocyte-derived macrophages by M-CSF in Man-MLV We also examined whether survival of monocyte-derived macrophages was affected by M-CSF in Man-MLV. For this, monocytes were incubated for 3 days in medium with 1,000 U/ml of M-CSF to allow their maturation and the resulting monocyte-derived macrophages were incubated for another 7 days in medium with free M-CSF (10 U/ml) or M-CSF encapsulated in control MLV or Man-MLV before assay of their survival. The results are shown in Table IV. Treatment of monocyte-derived macrophages with M-CSF in Man-MLV resulted in significant prolongation of their survival as compared to that of cells treated with free M-CSF or M-CSF in control MLV.

In a parallel experiment, we examined the abilities of monocyte-macrophages to phagocytose Man-MLV containing N-Rh-PE. For this, monocytes (1×10^6 cells/ml) were incubated for 7 days in medium with free M-CSF (1,000 U/ml). At the indicated times, these cells were

Table IV. Enhancement by Cetylmannoside Modification of MLV of Their Prolongation of Survival of Monocyte-derived Macrophages

Treatment[a]	Absorbance at 550 nm[b]	
	Medium	M-CSF (10 U/ml)
Medium	0.274±0.022	0.259±0.021
Control MLV (empty)	0.246±0.013	0.260±0.006
Man-MLV (empty)	0.252±0.011	0.243±0.023
Control MLV (M-CSF 10 U/ml)	0.301±0.045	0.273±0.025
Man-MLV (M-CSF 10 U/ml)	0.513±0.011[c]	0.489±0.010[d]

a) Blood monocytes were incubated for 3 days in medium with 1,000 U/ml of M-CSF, and the resulting monocyte-derived macrophages were then washed and incubated for another 7 days in medium with the indicated agents. Cell survival was measured as described in "Materials and Methods."
b) Mean±SD for triplicate cultures. Data are representative of three separate experiments.
c) Significantly different from the value for monocytes incubated in medium alone ($P<0.05$).
d) Significantly different from the value for monocytes incubated in medium with 10 U/ml of M-CSF alone ($P<0.05$).

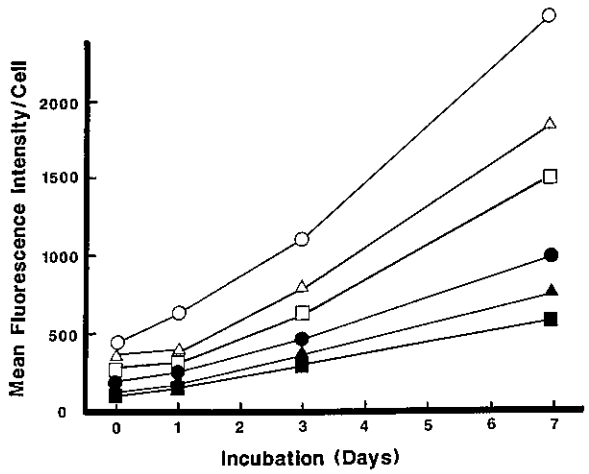

Fig. 4. Uptake of Man-MLV by monocyte-derived macrophages. Monocytes were incubated for the indicated times in medium with recombinant human M-CSF (1,000 U/ml), and then their abilities to take up control MLV(closed) or Man-MLV (open) containing N-Rh-PE at various concentrations of lipids of 250 nmol/ml (■, □), 500 nmol/ml (▲, △) and 1,000 nmol/ml (●, ○) were measured as described in "Materials and Methods." Points are mean values for 10,000 monocytes.

incubated for 3 h in CRPMI 1640 with control MLV or Man-MLV at various concentrations of lipids, and then washed and their fluorescence intensities were measured as described in "Materials and Methods." As shown in Fig. 4, monocyte-derived macrophages phagocytosed Man-MLV dose-dependently to greater extents than control MLV. Moreover, phagocytosis increased with maturation of the macrophages.

DISCUSSION

The present study demonstrates that modification of the liposome surface with cetylmannoside significantly increased the phagocytic uptake of the liposomes by blood monocytes over that of unmodified liposomes, and that BRMs encapsulated in cetylmannoside-modified MLV were far more effective than free BRMs or BRMs in unmodified MLV in augmenting monocyte functions (tumor cytotoxicity and survival potential).

Recently, much attention has been paid to the use of liposomes as carriers to deliver BRMs to cells of the mononuclear phagocyte system for treatment of primary and/or metastatic diseases.[1,2] The distribution and targeting of liposomal BRMs to specific tissues or organs seem to be dependent on the size, charge and composition of the MLV used.[1,23] In an attempt to develop an effective method for activating blood monocytes and macrophages in the liver, we focused on the mannose-binding sites expressed on monocyte-macrophages that allow their preferential interaction with mannose bound to carrier vehicles. Our previous study clearly showed that Man-MLV was preferentially delivered into the liver, presumably to Kupffer cells.[24]

Mannose receptors have been found to be expressed on mature macrophages, but not on fresh blood monocytes.[9-12,17] However, the present study showed that fresh monocytes phagocytosed Man-MLV better than unmodified MLV at 37°C (Fig. 1). Moreover, a high concentration of D-mannose, but not of D-galactose inhibited the uptake of Man-MLV, but not of control MLV by monocytes (Table I). Thus, the present findings indicate that mannose residues on the surface of MLV allow their direct interaction with mannose binding sites of fresh blood monocytes, resulting in the uptake of MLV by these cells. These findings are consistent with recent observations[18,21] showing that fresh blood monocytes possess mannose-6-phosphate receptors and/or lectin for D-mannose. The present findings together with previous reports,[1,2,25] show that norMDP encapsulated in the aqueous spaces of Man-MLV was far more effective than free norMDP for activating monocytes to the tumoricidal state (Fig. 2).

Previous studies showed that high concentrations of M-CSF alter the functions of human monocytes, prolonging their survival,[22] and inducing their cytotoxicity,[26] migration and Fc receptor expression.[27,28] In accordance with a previous report,[20] we found that relatively low concentrations of recombinant human M-CSF (more than 500 U/ml) prolonged monocyte survival for up to 21 days in culture. Interestingly, we also found that blood monocytes treated with M-CSF entrapped in Man-MLV survived for longer than those treated with free M-CSF (Fig. 3). It is noteworthy, however, that the absolute amount of M-CSF entrapped in Man-MLV was small. For example, when 1×10^4 U/ml of M-CSF was encapsulated in Man-MLV, 1000 nmol of Man-MLV contained 20 U of M-CSF in the aqueous spaces. Less than 50 U/ml of free M-CSF was not effective in prolonging monocyte survival, but the delivery of even 5 U/ml of culture medium of M-CSF to monocytes by Man-MLV resulted in significant prolongation of monocyte survival (Fig. 3). Moreover, anti-M-CSF antiserum did not affect prolongation of survival of monocytes by M-CSF in Man-MLV, suggesting that prolongation of survival is due to the delivery of M-CSF to the cytoplasm of monocytes, allowing its interaction with intracellular sites. At present, the mechanism by which M-CSF in Man-MLV prolongs monocyte survival is unknown.

The present findings showed that mature macrophages induced from monocytes by M-CSF were able to take up more Man-MLV than fresh monocytes. This result sup-

ports the idea of a close association of macrophage differentiation with mannose receptor expression.[12] Moreover, M-CSF entrapped in Man-MLV prolonged survival of monocyte-derived macrophages.

In this work, we found that modification of multilamellar liposomes with cetylmannoside increased their uptake by human blood monocytes, and that BRMs encapsulated in Man-MLV were far more effectively delivered to monocytes than free BRMs. These findings indicate the potential value of Man-MLV as carrier vehicles of BRMs for *in vivo* activation of human monocyte-macrophages to enhance the host's defense system against infections and cancer.

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