Activation of Tumoricidal Properties in Human Blood Monocytes by Liposomes Containing Lipophilic Muramyl Tripeptide

Eugenie S. Kleinerman, Kent L. Erickson, Alan J. Schroit, William E. Fogler, and Isaiah J. Fidler

ABSTRACT

Peripheral blood monocytes were isolated from normal human donors by separation on a continuous Percoll gradient and adherence to yield preparations of blood monocytes with a high degree of purity (>99%). The monocytes were incubated in vitro with medium alone or with multilamellar liposomes that contained either a lipophilic derivative of muramyl dipeptide, muramyl tripeptide (MTP-PE), or medium. The cytotoxic properties of the monocytes were assessed by an in vitro radioisotope release assay against various allogeneic targets. Monocytes that have phagocytosed liposomes containing MTP-PE were rendered tumoricidal. These monocytes lysed cells of three different tumorigenic lines but not cells of two nontumorigenic lines. The ability of MTP-PE-activated human blood monocytes to recognize and selectively lyse neoplastic cells was also demonstrated under cocultivation conditions. We conclude that human blood monocytes can be rendered tumoricidal after interaction with liposomes containing MTP-PE.

INTRODUCTION

Agents such as lymphokines and/or bacterial products can activate cells of the macrophage-histiocyte series to become tumoricidal (1, 3, 5, 10, 11, 13, 16–19). However, in vivo activation of macrophages by the injection of these agents has been only partially successful. One problem has been that free agents are not retained in the body long enough to activate macrophages to the tumoricidal state. For example, MDP, which is water soluble, is cleared from the body within 60 min of its parenteral administration (22), a time period not sufficient for it to render macrophages tumoricidal (13, 31, 32). MDP is the minimal structural unit of Mycobacteria (routinely used in Freund's adjuvant) that induces immunomodulating activity (2, 4, 18–20) and is now commercially available. Recent studies from our laboratory have shown that MDP (13, 31, 32) encapsulated within liposomes can be very efficient in rendering rodent macrophages tumoricidal both in vitro and in vivo. Moreover, the systemic administration of MDP encapsulated within MLV, unlike administration of free, unencapsulated material, leads to the eradication of established spontaneous pulmonary and lymph node metastases originating from a s.c. murine melanoma (13).

Recent studies have suggested that lipophilic derivatives of MDP that have acyl side chains covalently attached to the molecule are more effective than MDP for priming mouse peritoneal macrophages to release superoxide anions (21). Since low-molecular-weight compounds such as MDP can leak out of liposomes, we examined whether a lipophilic derivative of MDP, MTP-PE, inserted directly into the bilayer structure of MLV, would be as efficient for activation of human blood monocytes as it is for rodent macrophages (28). The ability of liposomes containing immunomodulators to activate tumoricidal properties in phagocytic human or rodent cells is interesting for 2 reasons: (a) elicitation of granulomas or hypersensitivity reactions can be avoided (10, 11); and (b) liposome-encapsulated agents have been shown to activate certain types of rodent tissue macrophages (histiocytes) that are refractory to activation by free agents (24).

In rodent systems, the distribution of liposomes in vivo (11) and their interaction with macrophages in vitro or in vivo are influenced by the size, chemical composition, and surface charge of the vesicles (10, 25, 26), and the ability of liposomes containing immunomodulators to activate rodent macrophages is well documented (8, 10, 11, 13, 25, 26, 28). Little comparable data on the interaction of human blood monocytes with liposomes are available. We have shown that human blood monocytes can rapidly phagocytose MLV composed of phosphatidylcholine and phosphatidylethanolamine admixed in a 7:3 mol ratio. Moreover, lymphokines derived from human lymphocytes entrapped within the aqueous interior of liposomes were very efficient in activating the cytotoxic properties of human blood monocytes. In this study, we wished to determine whether human blood monocytes could be activated similarly to the tumoricidal state by their interaction with MLV containing MTP-PE in the lipid bilayer. This issue is of considerable importance since, if successful, a synthetic immunomodulator such as MTP-PE could be used for the systemic activation of the reticuloendothelial system to improve the treatment of various diseases including cancer.

MATERIALS AND METHODS

Cell Cultures. A375 derived from a human melanoma was adapted to growth in culture as described previously (14, 34). HT-29 is a human colon carcinoma line, and the Natusch line is a human glioblastoma line. Both were a gift of Dr. Thomas Hoffman (NCI, Frederick Cancer Research Facility). Nontumorigenic human cell lines Flow 2000 derived from an embryonic lung and Flow 4000 derived from an embryonic kidney were purchased from Flow Laboratories, Rockville, Md. In our laboratory, the

1 Research supported in part by the National Cancer Institute, Department of Health and Human Services, under Contract N01-CO-23909 with Litton Bionetics, Inc.
2 Present address: Department of Human Anatomy, University of California, School of Medicine, Davis, Calif. 95616.
3 The abbreviations used are: MDP, muramyl dipeptide; MLV, multilamellar phospholipid vesicles; MTP-PE, muramyl tripeptide (N-acetylmuramyl-L-alanyl-2L-alanyl-2',1',3'-phosphorylglactosaminyl-L-alanyl-2-1'-dipalmityl-sn-glycero-3-phosphorylethanolamide); RPMI, Roswell Park Memorial Institute; HBSS, Hank's balanced salt solution.
3 tumor lines produced progressively growing tumors in nude mice, whereas the 2 nontumorigenic cell lines did not.

All cultures were free of Mycoplasma and were maintained on plastic in Eagle's minimal essential medium supplemented with 5% heat-inactivated fetal bovine serum, sodium pyruvate, and L-glutamine (M. A. Bioproducts, Walkersville, Md.) at 37° in a humidified atmosphere containing 5% CO₂. Cytotoxicity assays were performed when the cultured target cells were in their exponential growth phase.

**Reagents.** RPMI Medium 1640, human AB serum, fetal bovine serum, and HBSS were purchased from M. A. Bioproducts. Hydrophilic MDP and MTP-PE were the kind gift of Ciba-Geigy, Ltd., Basel, Switzerland. None of the reagents contained endotoxins as determined by the *Limulus* amebocyte lysate assay (sensitivity limit, 0.125 ng/ml).

Phospholipids and Preparations of Liposomes. Chromatographically pure egg phosphatidylcholine, dimyristoylphosphatidylethanolamine, and beef brain phosphatidylserine were purchased from Avanti Biochemicals, Birmingham, Ala. Phosphatidylcholine and phosphatidylserine were admixed at a 7.3 mol ratio and dissolved in chloroform. MTP-PE dissolved in methanol:chloroform (1:2) was added to the phospholipids.

After evaporation of the solvents and appropriate drying procedures, HBSS was added, and MLV were produced by mechanical agitation. The concentration of MTP-PE in the MLV ranged from 2 to 10 μg MTP-PE per μmol phospholipids. The incorporation of MTP-PE into the phospholipid bilayer membrane was confirmed by the ability of anti-MDP antibodies to specifically precipitate liposomes containing MTP-PE (30). Practically all (> 99%) of MTP-PE was incorporated into the liposomes as determined by studies utilizing radioactively labeled compound (30).

**Isolation and Culture of Human Monocytes from Mononuclear Blood Leukocytes.** Mononuclear blood leukocytes were collected from the peripheral blood of normal donors by separation on lymphocyte separation medium (LSM; Litton Bionetics, Kensington, Md.) and washed twice in HBSS. Peripheral blood monocytes were isolated from the leukocytes by further separation on a preformed continuous Percoll gradient as described previously (15). Briefly, the leukocytes (40 × 10⁶) were layered onto preformed Percoll gradients in 15-ml polycarbonate tubes and spun in swing-out buckets in a refrigerated centrifuge at 1000 × g for 20 min. Upon centrifugation, cell populations layered on top of the Percoll gradient separate on the basis of their relative densities into 2 distinct bands. The upper band is enriched for monocytes (80 to 90%) as determined by nonspecific esterase staining and morphological examination. The cells from this band were harvested, washed twice in HBSS, and then resuspended in RPMI Medium 1640 with 5% heat-inactivated human AB serum. After esterase staining, the suspension was adjusted to contain 1 × 10⁶ monocytes/ml. Monocytes (1 × 10⁶) were added to each well of a 96-well flat-bottomed Microtest II plate (Falcon Plastics, Oxnard, Calif.) that had been pretreated with fetal bovine serum for 1 hr at 37°. (The serum was removed before the addition of the cells, the target cells were prelabeled in vitro with either [3H]thymidine (40 Ci/mmol) or [3H]thymidine (40 Ci/mmol) purchased from New England Nuclear. The targets were A375 melanoma, HT-29 colon carcinoma, nontumorigenic lung fibroblasts, and nontumorigenic kidney cells. Radioactivity (0.5 μCi/ml medium) was added to the target cells in their exponential growth phase for 24 hr. The target cells were harvested as described above. Target cells were plated alone or in wells containing monocytes at an initial monocyte:target cell ratio of approximately 1:10. Radiolabeled monocytes were allowed to adhere for 1 hr at 37°. After incubation, the nonadherent cells were removed by washing with medium, and the plates were washed 3 times with RPMI Medium 1640. The plating efficiency of the monocytes was > 90%. The purity of monocytes at this point was > 99% as assessed by the ability of the cells to ingest carbon particles, examination of the cell morphology, and uptake of nonspecific esterase staining by the cells. Moreover, practically all the adherent cells stained positively with the monoclonal antibody 61D3 directed against human monocytes (Bethesda Research Laboratories, Bethesda, Md.).

**In Vitro Activation of Monocytes.** Monocytes were incubated at 37° in medium alone, medium containing various concentrations of MLV containing MTP-PE, or with MLV with entrapped HBSS which were suspended in medium with free MDP at a concentration equivalent to that entrapped within the MLV. Twenty-four hr later, the monocytes were washed thoroughly with medium before the addition of radiolabeled target cells.

**Monocyte-mediated Cytotoxicity.** Cytotoxicity was assessed by a modification of a radioisotope release assay described previously (27).

Target cells in exponential growth phase were incubated at 37° for 24 hr in the appropriate medium containing [3H]thymidazole (0.3 μCi/ml; specific activity, 200 nCi/μmol; New England Nuclear, Boston, Mass.). The cells were then rinsed twice to remove unbound radiolabel and harvested by a 1-min trypsinization with 0.25% Difco trypsin and 0.02% EDTA. The labeled cells were resuspended in medium with fetal bovine serum, and 10⁴ cells were plated into the culture well to obtain an initial target:effector cell ratio of approximately 1:10. Radiolabeled target cells were plated alone as an additional control group. The assay used here measures lysis of adherent target cells by adherent monocytes and requires direct cell-to-cell contact (1, 7, 10, 12, 16, 27). The cultures were washed 24 hr after the plating of tumor cells to remove all the nonadherent target cells, thereby providing a more accurate basis for analysis of actual monocyte-mediated lysis of target cells. In this system, the plating efficiency of target cells usually exceeds 80% whether the cells are plated alone, with control, or with activated monocytes. Time course studies revealed that target cell lysis (loss of radiolabel) began by 24 hr after plating and reached a maximum by 72 hr of cocultivation. For these reasons, after 24 hr of cocultivation, all cultures were aspirated to remove the nonplated cells, refed with fresh medium, and then cultured for an additional 2 days. At this time (72 hr), the cultures were washed twice with HBSS, and the adherent viable cells were lysed with 0.1 ml of 0.5 n NaOH. The radioactivity of the lysate was measured in a γ counter.

**Monocyte-mediated Cytotoxicity in Mixed Tumorigenic and Nontumorigenic Cultures.** In these experiments, tumorigenic and nontumorigenic human cell lines were cocultivated alone, with monocytes preincubated with 50 nmol of control liposomes (MLV containing HBSS and suspended in medium containing MDP), or with monocytes preincubated with 50 nmol of MLV containing a total of 0.25 μg MTP-PE. In order to distinguish between lysis of nontumorigenic and/or tumorigenic cells, the target cells were prelabeled in vitro with either [3H]thymidine (40 Ci/mmol) or [3H]thymidine (40 Ci/mmol) purchased from New England Nuclear. The targets were A375 melanoma, HT-29 colon carcinoma, nontumorigenic lung fibroblasts, and nontumorigenic kidney cells. Radioactivity (0.5 μCi/ml medium) was added to the target cells in their exponential growth phase for 24 hr. The target cells were harvested as described above. Target cells were plated alone or in wells containing monocytes at an initial monocyte:target cell ratio of 10:1. The cultures were treated as described above and harvested after 3 days of cocultivation. At this time, the cultures were washed 3 times with warm HBSS to remove any nonadherent cells. The remaining adherent cells were lysed with 0.5 n NaOH, and the lysate was placed into scintillation vials containing Aquasol II (New England Nuclear). Radioactivity was monitored in a Beckman scintillation counter.

**Calculation of Percentage of Monocyte-mediated Cytotoxicity.** For all experiments, the percentage of cytotoxicity was calculated according to the formula

\[
\% \text{ of generated cytotoxicity} = \frac{\text{cpm in target cells cultured with control monocytes} - \text{cpm in target cells cultured with test monocytes}}{\text{cpm in target cells cultured with control monocytes}} \times 100
\]

Statistical Analysis. Experimental results were analyzed for their statistical significance by Student’s t test (2-tailed).

**RESULTS**

Activation of Tumoricidal Properties in Human Blood Monocytes by MTP-PE in Liposomes. The studies described here used highly homogeneous (> 99%) preparations of blood monocytes devoid of spontaneous cytotoxicity. This lack of reactivity is primarily attributed to the procedure of monocyte harvest but also may be because the reagents were not contaminated with
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endotoxins. Monocytes incubated in medium as well as those pretreated with MLV containing HBSS and suspended in 2 μg of free MDP (“out” control) did not significantly lyse the A375 target cells. The incubation of monocytes with liposomes containing MTP-PE led to the generation of cytotoxic properties (Table 1). The results we obtained in 3 independent experiments were quite similar. Monocytes were also treated with different concentrations of MLV ranging from 25 to 200 nmol phospholipids per culture well containing 10⁶ monocytes. Different preparations of liposomes contained varying amounts of MTP-PE, ranging from 25 to 10 μg MTP-PE per μmol phospholipid. By using this double-dose response study, we determined that the optimal activation of human blood monocytes required the delivery of 0.25 to 0.5 μg of MTP-PE encapsulated in 50 to 100 nmol of MLV to 10⁶ monocytes (Table 1). In all the subsequent experiments, monocytes were activated by a 24-h incubation with 50 nmol MLV containing 0.25 μg MTP-PE. MLV containing HBSS within their aqueous interior and suspended in an amount of unencapsulated MDP calculated to be equivalent to that entrapped within MLV did not render the monocytes tumoricidal. This indicated that the activation of monocytes by MLV required the internalization of MTP-PE.

The effects of free (unencapsulated) MTP-PE on activation of blood monocytes are difficult to assess because the material is not water soluble. For this reason, we used hydrophilic MDP (in equivalent amount) for all control experiments (the “out” control). Dose-response studies determined that the maximal in vitro activation of human blood monocytes required 20 to 40 μg of free hydrophilic MDP per ml medium (data not shown). In contrast, activation of monocytes to similar levels of tumor cytotoxicity by MDP in liposomes was achieved with as little as 25 to 40 ng of the agent per culture (data not shown). Thus, in this human system, liposome-encapsulated MDP activated blood monocytes approximately 1000 times more efficiently than did free MDP. These data are very similar to those found in rodent systems (28, 29, 31, 32). Liposomes containing MTP-PE with a dose of 60 ng of the agent per culture significantly activated monocytes (Table 1). This dose is approximately 1/20th the dose of free MDP required to achieve similar levels of monocyte activation. Moreover, even when the doses of MDP and MTP-PE are calculated on a mol equivalent basis, MTP-PE (M, 1269) is approximately twice as efficient in rendering monocytes tumoricidal as is encapsulated MDP (M, 495).

Spectrum of Monocyte-mediated Cytotoxicity. In the next set of experiments, we examined whether monocytes activated by MTP-PE in MLV are able to lyse selectively only tumorigenic cells in vitro. One representative experiment of 4 is shown in Table 2. Control monocytes treated with medium were not cytotoxic against any of the tumorigenic or nontumorigenic targets. Human blood monocytes treated in vitro with MLV containing MTP-PE were cytolytic to allogeneic A375 melanoma cells, HT-29 colon carcinoma cells, and Natusch glioblastoma cells but did not lyse the allogeneic nontumorigenic kidney or lung cells.

Specificity of Tumorogenic Monocytes under Cocultivation Conditions. To further address the issue of target specificity and to determine whether nontumorigenic cells could be lysed as "innocent bystanders," we examined the ability of monocytes activated by MTP-PE to discriminate between tumor and normal cells under cocultivation conditions. Tumorogenic A375 melanoma or HT-29 colon carcinoma cells and nontumorigenic kidney or lung cells were prelabeled with either [³H]thymidine or [¹⁴C] thymidine. Either 10⁴ target cells (when cultured alone) or 5 x 10⁵ target cells (when cultured in combinations) were plated alone or with control or tumoricidal monocytes (Table 3). Control monocytes did not demonstrate detectable levels of spontaneous cytotoxicity against either tumorigenic or nontumorigenic target cells. Monocytes treated with liposomes containing MTP-PE lysed tumorigenic A375 melanoma and HT-29 carcinoma cells but left the lung or kidney cells unharmed. In all the combinations, monocytes activated in vitro with MLV containing MTP-PE were highly cytotoxic against neoplastic cells but not against nonneoplastic cells regardless of which isotope was used to label the cells. Furthermore, when the 2 different tumorigenic target cell lines were cocultivated with tumoricidal monocytes, release of both isotopes was observed. In contrast, neither radiolabel was released from cocultivated nontumorigenic target cell lines.

DISCUSSION

Our studies demonstrate that human blood monocytes can be activated to become tumoricidal after their interaction in vitro with MLV that contain a lipophilic derivative of MDP. MDP has been shown to influence many functions of macrophages such as the production of prostaglandins and collagenases, capacity to generate O₂-, proliferation in response to lymphokines, and motility and cytotoxic activity against neoplasms (19). In rodent systems, macrophages are activated to the tumoricidal state only after long incubations with free MDP (30), suggesting that activation involves a slow passive uptake mechanism into the cells as opposed to a receptor-mediated transport system such as that observed for lymphokines (10, 25). This finding and the demonstration that > 90% soluble MDP is excreted from the

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose of MLV (nmol lipid/culture)</th>
<th>Dose of MTP-PE (μg/ml)</th>
<th>Residual radioactivity in adherent target cells (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>200</td>
<td>10</td>
<td>2939 ± 450 (34)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1</td>
<td>1890 ± 354 (58)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.5</td>
<td>1777 ± 338 (60)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.25</td>
<td>2140 ± 530 (50)</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>5</td>
<td>2243 ± 286 (50)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.5</td>
<td>1224 ± 300 (72)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.25</td>
<td>1754 ± 306 (61)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.125</td>
<td>2296 ± 359 (49)</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.5</td>
<td>3663 ± 265 (19)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.25</td>
<td>3612 ± 218 (20)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.125</td>
<td>3303 ± 306 (26)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.06</td>
<td>3100 ± 577 (30)</td>
</tr>
</tbody>
</table>

* Radioactivity in adherent viable cells after 72-hr cultivation.
* Mean ± S.D. of triplicate cultures.
* Numbers in parentheses, percentage of generated cytotoxicity calculated by comparison to cultures with monocytes treated with MLV containing medium.
* p < 0.01.
* p < 0.001.
* Control culture of MLV containing medium and suspended in free MDP.

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body within 60 min of administration (22) may explain the failure of soluble MDP to activate macrophages in situ.

Several methods have been proposed to enhance the in vivo immunomodulating activity of MDP. One that we have explored in our laboratory is the use of liposomes as vehicles for the transport of compounds into phagocytic cells. MLV are attractive vehicles for the delivery of biologically active materials because phagocytosed MLV release the agent intracellularly over a sustained period of time (28). Indeed, MDP or MTP-PE contained in MLV activate rodent macrophages very efficiently in vitro and in vivo (29). In these studies, we have shown that MTP-PE encapsulated in MLV composed of phosphatidylcholine and phosphatidylserine also rendered human blood monocytes tumoricidal. MTP-PE encapsulated in liposomes is superior to liposome-encapsulated MDP for activation of macrophages in situ (28).

Water-soluble MDP, like most soluble agents, can easily leak out of encapsulated MDP for activation of macrophages in situ (28). In contrast, MDP or MTP-PE contained in vehicles for the delivery of biologically active materials because soluble MDP to activate macrophages in situ.

Table 2

Tumoricidal activity of human blood monocytes activated in vitro by MTP-PE in liposomes

Blood monocytes (10^6) were treated as indicated for 24 hr before the addition of 10^6 [3H]iododeoxyuridine-labeled target cells. Radioactivity in viable cells was determined after 72 hr of cultivation.

<table>
<thead>
<tr>
<th>Target cells</th>
<th>Target cells alone</th>
<th>Monocytes incubated in medium</th>
<th>Monocytes treated with control MLV</th>
<th>Monocytes treated with MTP-PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumorigenic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A375 melanoma</td>
<td>1801 ± 95</td>
<td>1764 ± 93</td>
<td>1615 ± 70</td>
<td>1086 ± 145 (32)</td>
</tr>
<tr>
<td>HT-29 colon</td>
<td>1359 ± 28</td>
<td>1464 ± 63</td>
<td>1500 ± 121</td>
<td>681 ± 18 (55)</td>
</tr>
<tr>
<td>Natusch</td>
<td>2978 ± 223</td>
<td>2808 ± 105</td>
<td>2679 ± 151</td>
<td>1767 ± 152 (36)</td>
</tr>
<tr>
<td>Nontumorigenic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung cells</td>
<td>1727 ± 26</td>
<td>1668 ± 49</td>
<td>1752 ± 116</td>
<td>1787 ± 28</td>
</tr>
<tr>
<td>Kidney cells</td>
<td>1422 ± 54</td>
<td>1433 ± 160</td>
<td>1466 ± 60</td>
<td>1462 ± 151</td>
</tr>
</tbody>
</table>

| a | Fifty nmol of MLV containing medium and 1 µg free MDP per culture.  
| b | Fifty nmol of MLV containing 0.25 µg of MTP-PE.  
| c | Mean cpm ± S.D. for triplicate cultures.  
| d | Numbers in parentheses, percentage of generated cytotoxicity calculated by comparison to cultures with monocytes treated with MLV containing medium.  
| e | p < 0.005.

Table 3

Recognition and lysis of neoplastic cells by tumoricidal human blood monocytes (cocultivation experiments)

Blood monocytes (10^6) were incubated with either medium (control) or with 50 nmol of MLV containing 0.25 µg MTP-PE for 24 hr before the addition of radiolabeled target cells. A total of 10^6 cells labeled with either [3H]thymidine (0.5 µCi/ml) or [14C]thymidine were plated alone or with the monocytes. In mixed-population experiments, 5 x 10^3 cells of each target were added into each culture well.

<table>
<thead>
<tr>
<th>Target cells and radioactivity</th>
<th>Target cells alone</th>
<th>Target cells with control monocytes (cpm)</th>
<th>Target cells with tumoricidal monocytes (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A375 melanoma, [3H]Thd</td>
<td>47,719 ± 668</td>
<td>47,269 ± 830</td>
<td>27,185 ± 843 (42%)</td>
</tr>
<tr>
<td>HT-29 carcinoma, [3H]Thd</td>
<td>35,341 ± 871</td>
<td>35,341 ± 469</td>
<td>14,802 ± 256 (58)</td>
</tr>
<tr>
<td>Lung cells, [3H]Thd</td>
<td>28,847 ± 357</td>
<td>28,341 ± 1,153</td>
<td>29,089 ± 472 (472)</td>
</tr>
<tr>
<td>Kidney cells, [3H]Thd</td>
<td>37,936 ± 502</td>
<td>38,595 ± 299</td>
<td>37,901 ± 549 (949)</td>
</tr>
<tr>
<td>A375 melanoma, [3H]Thd, and lung cells, [3C]Thd</td>
<td>18,954 ± 1,019</td>
<td>21,564 ± 543</td>
<td>11,213 ± 414 (48)</td>
</tr>
<tr>
<td>HT-29 carcinoma, [3H]Thd, and lung cells, [3C]Thd</td>
<td>20,157 ± 6,277</td>
<td>21,228 ± 530</td>
<td>11,463 ± 241 (46)</td>
</tr>
<tr>
<td>HT-29 carcinoma, [3H]Thd, and lung cells, [3C]Thd</td>
<td>17,974 ± 493</td>
<td>18,237 ± 526</td>
<td>8,753 ± 184 (52)</td>
</tr>
<tr>
<td>A375 melanoma, [3H]Thd, and kidney cells, [3C]Thd</td>
<td>18,666 ± 617</td>
<td>18,901 ± 741</td>
<td>9,072 ± 450 (52)</td>
</tr>
<tr>
<td>HT-29 carcinoma, [3H]Thd, and kidney cells, [3C]Thd</td>
<td>20,398 ± 527</td>
<td>19,986 ± 613</td>
<td>20,233 ± 503 (503)</td>
</tr>
<tr>
<td>Lung cells [3H]Thd, and kidney cells, [3C]Thd</td>
<td>18,115 ± 423</td>
<td>18,426 ± 339</td>
<td>10,084 ± 400 (45)</td>
</tr>
</tbody>
</table>

| d | dThd, thymidine.  
| e | Mean ± S.D. of triplicate cultures.  
| f | Numbers in parentheses, percentage of cytotoxicity as compared with control monocytes and target cells (p < 0.001).
not destroy nontumorigenic cells even under conditions of co-cultivation (Table 3). The mechanism for this selective lysis, however, is still unknown.

A growing body of data demonstrates that rodent and human tumors are heterogeneous and contain subpopulations of cells with different biological characteristics (6, 9, 23). Recently, we have demonstrated that some metastases are clonal in origin and that different metastases result from the proliferation of different progenitor cells (33). In part, this may explain the finding that different metastases exhibit different responses to conventional therapeutic agents (34). At least in vitro, activated rodent (12) and human (1, 17) (Tables 2 and 3) macrophages are able to recognize and lyse many tumorigenic cells regardless of such cell characteristics as metastatic potential and susceptibility to cytotoxic agents. Therefore, the in situ activation of tumoricidal properties in macrophages may provide an attractive approach for treatment of metastases resistant to conventional therapeutic agents. Indeed, the activation of tumoricidal properties in macrophages of mice bearing metastases by systemic administration of liposomes containing MDP has been shown to have significant therapeutic benefits (8, 13). Because human blood monocytes interact in vitro with liposome-encapsulated MTP-PE in a manner similar to that of rodent macrophages, the present studies suggest that further trials on the use of liposome-encapsulated MTP-PE for activation of human macrophages in vitro and eventually in vivo are warranted.

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