Maltose Tetrapalmitate, a Nontoxic Immunopotentiator with Antitumor Activity

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ABSTRACT

The attempt to synthesize a lipid A-like component (the active portion of lipopolysaccharides, but lacking its endotoxic activity) resulted in the production of fatty acyl sugars of which maltose tetrapalmitate was seen to yield the most promising results. It shows no endotoxic activity and elicits an antitumor response in tumor-transplanted animals as shown by (a) an enhancement of the host's capacity to reject a large number of tumor cells, (b) retardation of growth in tumor size, and (c) induction of hemorrhagic necrosis in certain tumors. Experiments with mammary ascites carcinoma show maltose tetrapalmitate to be as effective as is bacterial glycolipid mRS95 in its antitumor activity. The degree of sensitivity to maltose tetrapalmitate varies with the tumor-host system: mammary ascites carcinoma < NH = ClT,TS,V, < S = B16 < L26. The mode of action of maltose tetrapalmitate appears to be via its modulation of the immune system. It is itself noncytotoxic to tumor cells in vitro. It is seen to stimulate the spleen cells of certain animals mitogenically, although it causes tumor rejection in all the types of animals tested. Also, it activates peritoneal exudate macrophages in tumor-bearing animals; whether specifically or nonspecifically has not yet been established.

INTRODUCTION

It is well known that endotoxic bacterial LPS* possess immunological reactivities such as mitogenic stimulation of B-lymphocytes (2), macrophage activation (1), replacement of helper T-cell function during antibody production against antigens in vivo (15), and conversion of a tolerogenic dose of an antigen to an immunogenic dose (8). In high doses LPS are also known to be immunosuppressive (9). The former features (1, 2, 8, 15) of the LPS molecule make them useful substances in the immune rejection of tumors. However, their endotoxic activity prevents their application in human immunotherapy despite their known antitumor potential against certain transplanted tumors (10, 11). Lipid A is generally considered to be the active part of the LPS molecule that elicits immunological reactivity and possesses endotoxic activity.

Our purpose in this study was to synthesize a lipid A-like molecule that would be immunologically reactive and devoid of endotoxic activity so that it could be tested for its antitumor activity. Antitumor activity in such a molecule would suggest that (a) enhancement of immune response can cause tumor rejection and (b) endotoxic activity need not accompany immune reactivity or tumor rejection ability.

During the past 3 years we have synthesized several glycolipid esters by fatty acylation of simple carbohydrates and nucleosides. These are termed “synthetic glycolipids.” Those derived by O-esterification of maltose, cellobiose, and D-galactose with palmitoyl chloride were found to enhance serum antibody titers eight-fold in rabbits against SRBC used as an antigen (unpublished data). They were also mitogenic for spleen lymphocytes of Swiss mice in vitro. One of the glycolipids derived from maltose has been tentatively characterized as MTP and has been subjected to investigation for its antitumor activity, for its ability to act as an immunopotentiator, and for its toxicity. This paper will describe the results of this investigation and, briefly, the synthesis of this compound.

MATERIALS AND METHODS

Synthesis of MTP. Dry maltose (10 mmol) was dissolved in 40 ml of dry dimethylformamide at 60°, and 5 ml of dry pyridine were then added. To the mixture a solution of palmitoyl chloride (40 mmol) in 5 ml dry dimethylformamide was added dropwise with stirring. The mixture was left at 60° for 4 to 5 hr and then left at room temperature. It was subjected to thin-layer chromatography on Silica Gel G plates with CHCl₃:CH₃OH:H₂O (60:25:4) as the developing solvent. The plates were stained with resorcinol reagent (20). Six bands with Rₗ's of 0.95, 0.80, 0.68, 0.54, 0.39, and 0.23 were identified. The Rₗ 0.68 band occurred in the largest amount. This glycolipid was isolated by chromatography on a Silica Gel G column (3.8 x 40 cm) with the solvent mixture described above. A yield of 200 to 300 mg was obtained. On hydrolysis (4 N HCl for 4 hr at 100°), it gave a mixture of glucose and palmitic acid in the approximate ratio of 1:2, both determined colorimetrically (5, 18). Carbon and hydrogen analyses gave the following result for MTP.

\[ C_{98}H_{140}O_{15} \]

Calculated: C 70.5, H 11.09
Found: C 69.92, H 11.15

The compound was nonreducing, indicating that C-1 was esterified. Periodate oxidation of the compound followed by sodium borohydride reduction and mild acid hydrolysis (18) failed to reveal the presence of glucose upon paper chromatographic analysis of the reaction mixture. This indicates that both glucose residues of maltose contain a pair of adjacent hydroxyl groups in the MTP molecule. The


* The abbreviations used are: LPS, lipopolysaccharides, SRBC, sheep red blood cells; MTP, maltose tetrapalmitate; MAC, mammary ascites carcinoma; RPMI, Roswell Park Memorial Institute; PFC, plaque-forming cells; PEC, peritoneal exudate cells; l.t., intratumorally; BCG, Bacillus Calmette-Guérin.

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compound is suggested to be either 1,6,2',6'- or 1,6,4',6'-tetra-O-palmitoyl maltose.

**Tumors and Antitumor Activity Determination.** The transplantable tumors used for the determination of antitumor activity of MTP, with their source given in parentheses, were as follows: MAC 13762 in Fischer 344/CRBL rats (Dr. A. E. Bogden, Mason Research Institute, Worcester, Mass.); ascites form of Novikoff hepatoma in Sprague-Dawley rats (Dr. R. Morais, Institut du Cancer de Montréal, Montreal, Canada); cultures of a SV40-induced sarcoma (Cl, TSV,S) in Syrian hamsters (Dr. P. Tournier, Centre National de Recherche Scientifique, Villejuif, France); line 26 colon tumor (L26) in BALB/c mice (Dr. T. H. Corbett, Southern Research Institute, Birmingham, Ala.,); and B16 melanoma (B16) in C57BL/J mice (The Jackson Laboratory, Bar Harbor, Maine). The required tumor recipients were purchased from Charles River Breeding Co., St. Constant, Quebec, Canada, and from The Jackson Laboratory.

A known number of tumor cells or a piece of tumor of known size was transplanted s.c. above the right thigh of the animals on Day 0, and animals were treated with 10 μg MTP suspension in 0.3 ml 0.9% NaCl solution per animal s.c. on the left thigh on Day 1 and then every third day until the termination of the experiment. Controls received 0.9% NaCl solution alone. The number of animals with and without tumor and the tumor size were recorded. In other experiments tumors were allowed to grow to approximately 1 cu cm, and MTP treatment was begun and continued as described previously.

**Toxicity Determination.** The toxicity of MTP was determined in the chick embryo lethality test (16) and the endotoxic activity by the Limulus lysate assay (7). Groups of Swiss mice were also treated i.p. with an increasing dose of MTP up to 10 mg/mouse, and the number of animals that died within 48 hr were recorded.

**Immunological Techniques.** Isolation of spleen lymphocytes and their stimulation by MTP were carried out by techniques similar to those described by Weinstein et al. (21). RPMI Medium 1640 containing 10% heat-inactivated fetal bovine serum was used throughout the investigation. PFC were determined according to the method of Jerne et al. (6), 5 days after an i.p. injection of sheep RBC was given to rats. Serum anti-sheep RBC antibody titters were determined according to Beckmann et al. (3), 10 days after 10⁶ sheep RBC injection i.p. was given to rats. Antibody titters against tumor cells were determined by the complement-fixation technique (12).

PEC were isolated by lavage of the peritoneal cavity with phosphate-buffered saline, 2 days after a 10-ml injection of mineral oil to rats had been given. Washed oil-free PEC were suspended in RPMI Medium 1640 containing 10% fetal bovine serum. For in vitro activation of PEC by MTP, 10⁶ PEC in 0.2 ml medium were added to a number of microtiter plates, one-half of which contained 5 μg MTP in 10 μl 0.9% NaCl solution. They were incubated for 48 hr in a CO₂ incubator (95% air-5% CO₂). The medium was then removed, and to the attached cells 10⁶ MAC cells in 0.2 ml medium were added. After 3 days of incubation (³H)thymidine (1 mCi in 10 μl) was added to all the wells, and 18 hr later cellular DNA was precipitated by acid and counted for its ³H. Controls consisted of untreated and MTP-treated PEC and MAC alone. Percentage of cytotoxicity was used as a measure of in vitro stimulation of PEC by MTP and was given by

\[
100 - \left( \frac{\text{cpm in MAC-PEC} - \text{cpm in PEC}}{\text{cpm in MAC}} \right) \times 100
\]

For determination of MTP-mediated in vivo activation of PEC and spleen lymphocytes that could render them cytotoxic to injected tumor (MAC or Cl, TSV,S) cells, 4 groups of animals (rats or hamsters) each were treated with 0.5 ml 0.9% NaCl solution i.p., 10 μg MTP i.p., and 10⁶ tumor cells s.c. The animals received injections i.p. of 10 ml mineral oil 4 days later. After 3 days animals were sacrificed and their PEC and spleen cells were isolated. Cytotoxicities of rat and hamster spleen lymphocytes and PEC against target MAC and Cl, TSV,S, respectively, were determined. An unrelated tumor cell line (spontaneously transformed Wistar rat fibroblasts) was used as a nonspecific tumor target described against rat PEC.

**Materials.** Maltose was purchased from Sigma Chemical Co., St. Louis, Mo., and palmitoyl chloride was from Eastman Kodak, Rochester, N. Y. All other chemicals were obtained from Fisher Scientific Co., Montreal, Canada. RPMI Medium 1640 and fetal bovine serum were purchased from Flow Laboratories, Rockville, Md. Bacterial glycolipid mR595 was a gift from Dr. Otto Luderitz, Max-Planck Institut für Immunobiologie, Freiburg, West Germany. Nude mice of BALB/c background were raised in our departmental animal house facilities.

**RESULTS**

**Toxicity of MTP.** LD₅₀ of MTP in Swiss mice could not be determined because quantities were limited and amounts of MTP as large as 10 mg/mouse injected i.p. failed to cause mortality of even 1 mouse in a group of 10. Bacterial endotoxin (mR595) gave a dose lethal to 50% of mice of 400 μg in the same test. Similarly, in the chick embryo lethality test, 100 μg of MTP were nontoxic. The comparative figure for the dose lethal to 50% mice of mR595 was 0.007 μg. In the Limulus lysate assay, 100 μg MTP per ml failed to induce gel formation, whereas 1 mg MTP/ml caused a thickening of the lysate. In this assay mR595 caused gel formation at a concentration of 0.001 μg/ml.

**Antitumor Activity of MTP against Transplanted Tumors.** MTP was tested for its antitumor activity in 5 transplanted animal tumor systems in 3 species of animals, as described in "Materials and Methods." In addition the effect on the growth of Cl, TSV,S in nude mice by MTP was also tried. An optimum MTP dose of 10 μg/animal was selected for all experiments after a preliminary screening of the dose-response relationship with 2 tumor-host systems (MAC and Cl, TSV,S) indicated that, although a dose as low as 0.1 μg MTP per animal sometimes retarded increase in tumor size, consistent results were obtained with 10 μg per animal. Subsequent work confirmed this finding (see later). The results of the experiment are shown in Table 1. It was observed that insofar as tumor development by a known and limited number of inoculated tumor cells was concerned, MTP was effective in reducing the number of tumor takes in all of the 3 tumors used (MAC, NH, and Cl, TSV,S).

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Table 1

Antitumor activity of MTP against transplanted tumors

For details, see "Materials and Methods."

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Recipient</th>
<th>Experiment</th>
<th>No. of cells transplanted or tumor size</th>
<th>Treatment</th>
<th>Animals without tumor/total no. of animals at 21-24 days</th>
<th>Survival times of animals with established tumor (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAC</td>
<td>Fischer 344 rats</td>
<td>1</td>
<td>5 x 10^6</td>
<td>0.9% NaCl solution</td>
<td>0/5 (2.3 ± 0.2)</td>
<td>23.9 ± 5.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>5 x 10^6</td>
<td>MTP</td>
<td>2/5 (1.9 ± 0.1)</td>
<td>31.6 ± 4.7</td>
</tr>
<tr>
<td>NH</td>
<td>Sprague-Dawley rats</td>
<td>1</td>
<td>1 x 10^6</td>
<td>0.9% NaCl solution</td>
<td>1/9 (4.1 ± 0.5)</td>
<td>28.5 ± 5.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1 x 10^6</td>
<td>MTP</td>
<td>8/24 (2.1 ± 0.3)</td>
<td>45.1 ± 6.2</td>
</tr>
<tr>
<td>Cl_TSV_S</td>
<td>Syrian hamsters</td>
<td>1</td>
<td>1 x 10^6</td>
<td>0.9% NaCl solution</td>
<td>4/9 (4.0 ± 0.4)</td>
<td>62.2 ± 6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1 x 10^6</td>
<td>MTP</td>
<td>8/9 (2.6 ± 0.3)</td>
<td>84.0 ± 7.2</td>
</tr>
<tr>
<td>L26</td>
<td>BALB/c mice</td>
<td>1</td>
<td>8 cu mm</td>
<td>0.9% NaCl solution</td>
<td>1/4 (2.0 ± 0.1)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>8 cu mm</td>
<td>MTP</td>
<td>11/12 (1.5)</td>
<td></td>
</tr>
<tr>
<td>B16</td>
<td>C57BL/J mice</td>
<td>1</td>
<td>8 cu mm</td>
<td>0.9% NaCl solution</td>
<td>0/4 (2.5 ± 0.3)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>8 cu mm</td>
<td>MTP</td>
<td>1/4 (2.0 ± 0.2)</td>
<td></td>
</tr>
<tr>
<td>Cl_TSV_S</td>
<td>Nude mice</td>
<td>1</td>
<td>1 x 10^6</td>
<td>0.9% NaCl solution</td>
<td>0/6 (6.0 ± 0.4)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 x 10^6</td>
<td>MTP</td>
<td>1/10 (3.5 ± 0.3)</td>
<td></td>
</tr>
</tbody>
</table>

a Numbers in parentheses, tumor size (sq cm).
b Antibody titers in tumor-bearing animals were as follows: Anti-MAC, 0.9% NaCl solution-treated 1 to 1/2, MTP-treated 1/2 to 1/4 without tumor 1/4 to 1/16; Anti-NH, 0.9% NaCl solution-treated 1/2 to 1/16, MTP-treated 1/16 to 1/32, without tumor 1/64 to 1/1024; anti-Cl_TSV_S, 0.9% NaCl solution-treated 1/2 to 1/4, MTP-treated 1/4 to 1/8.
c Mean ± S.D.
d The differences between untreated and MTP-treated animals are statistically nonsignificant.
e ND, not done.
f Tumors were hemorrhagically necrotized.

Moreover, the size of tumors that developed in MTP-treated animals was smaller than that of 0.9% NaCl solution-treated controls. Among these 3 tumors MAC was the least sensitive to MTP treatment, a 2-fold increase in the number of tumor cells injected (from 5 x 10^6 to 1 x 10^7 cells) abolished the ability of MTP to induce rejection of tumor cells, and the tumors that developed were of the same size as those observed in the control animals. In contrast to MAC MTP was much more effective against Cl_TSV_S tumor in hamsters. The viable cell inoculum for 50% tumor takes given 4 and 5 weeks after tumor inoculation, was found to be 2 x 10^3 cells in the case of 0.9% NaCl solution-treated control animals and 1 x 10^5 cells in the case of animals treated with MTP (Chart 1). Thus, MTP treatment enhanced the ability of the animals to reject a 5000-fold higher tumor cell dose.

The solid tumors (L26 and B16) transplanted s.c. via trocar were also sensitive to MTP-induced tumor rejection. Thus, whereas 1 of 4 animals transplanted with L26 rejected the tumor transplant on 0.9% NaCl solution treatment, 11 of 12 rejected tumor on MTP treatment. In the case of B16, MTP treatment resulted in the rejection of the tumor in about 20% of the mice in 2 experiments compared to no rejection in animals kept on 0.9% NaCl solution treatment. In both of these cases, the animals that failed to reject the tumor, those treated with MTP bore smaller tumors than did 0.9% NaCl solution-treated controls.

The effect of MTP treatment on the survival times of animals that bear growing MAC, NH, and Cl_TSV_S tumors is shown in Table 1. Treatment began when the tumor had reached a size of approximately 1 cu cm. It was observed that MTP did not cause rejection of these large tumors and that the survival times of animals bearing MAC, NH, and Cl_TSV_S tumors were insignificantly increased.

When nude mice were given transplants of a xenograft of Cl_TSV_S cells and MTP treatment was instituted, the tumor cells were not rejected but the size of tumors that developed was smaller in the MTP-treated group than in the 0.9% NaCl solution-treated controls (Table 1).

Since MAC was the least sensitive to s.c. MTP injection away from the tumor site, we tried i.t. injection of MTP in MAC-bearing animals. Neither a rejection of the tumor nor a decrease in tumor size was observed. Further, when MTP was injected on Day -7, -3, and 0 and the animals were inoculated with 10^6 MAC, the tumor cells were not rejected,
and neither was the size of tumors that developed in MTP-pretreated animals smaller than in the controls.

**MTP Dose and Antitumor Response Relationship.** Chart 2 shows the number of tumor takes and the size of tumors that developed in response to treatment with various doses of MTP in MAC, CTmSV, and B16 tumor-host systems. In the case of MAC, it was observed that a MTP dose of 5 µg or higher was adequate in preventing tumor appearance in a maximum number (40%) of the animals and in keeping the size of the tumor to a minimum, which was 40% smaller than in the controls. With CTmSV tumor, minimum tumor takes were with a dose of 10 µg MTP. The number of animals with tumor, with this dose, was 30% compared to 80% in the controls. The size of tumors that developed was about the same in animals treated with 0.1 to 50 µg MTP, but it was 40 to 50% smaller in MTP-treated animals as compared to controls. Results with B16 indicated that a MTP dose of 10 µg was the most effective, giving 20% less tumor takes as compared to 100% in 0.9% NaCl solution-treated controls. The size of the tumor was reduced maximally (about 40%) when the MTP dose range was between 1 and 50 µg.

**Action of MTP on the Immune System.** On the basis of the known action of LPS on various arms of the immune system of the animals, we tested whether MTP (a) was a mitogen for spleen lymphocytes of Fischer rats, nude mice, and mice of various strains; (b) was capable of enhancing serum antibody titers and spleen PFC with SRBC used as an antigen; and (c) could render in vitro peritoneal macrophages cytotoxic to tumor cells. The results are shown in Table 2. It was found that MTP was mitogenic for spleen lymphocytes of Fischer rats, Swiss mice, AKR mice, and nude mice. It did not mitogenically stimulate spleen lymphocytes of C57BL/J mice and Syrian hamsters. Mitogenic stimulation of spleen cells was found to peak at 1 to 2 µg MTP per ml in the responding species and strains of animals. High concentrations of MTP (50 µg/ml) inhibited the background counts of [3H]thymidine incorporated into DNA as well as phytohemagglutinin-mediated stimulation of spleen lymphocytes (not shown).

MTP treatment of Fischer rats, following i.p. SRBC inoculation, showed a 2- to 4-fold increase in spleen PFC on Day 4 that depended on the number of SRBC injected (Table 2). The anti-SRBC serum antibody titers were increased 8-fold on Day 7 in MTP-treated animals (Table 2). In vitro MTP treatment was also effective in activating peritoneal macrophages of Fischer rats so that they were cytotoxic for MAC (Table 2).

In a test of whether in vivo MTP treatment rendered peritoneal macrophage and spleen cells cytotoxic to tumors cells as well, groups of rats and hamsters were (a) inocul...
Table 2

**Immunological reactivities of MTP**

<table>
<thead>
<tr>
<th>Spleen lymphocytes of</th>
<th>Addition</th>
<th>Mitogenic stimulation of spleen lymphocytes in vitro</th>
<th>Anti-SRBC PFC and antibody responses on Fischer rats</th>
<th>Activation of Fischer rat peritoneal macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>[³H]Thymidine incorporation (cpm/10⁶ cells)</td>
<td>PFC response with 5 × 10⁶ SRBC</td>
<td>1 × 10⁷ SRBC</td>
</tr>
<tr>
<td>Swiss mice</td>
<td>None</td>
<td>1341 ± 110⁷</td>
<td>None</td>
<td>10 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>MTP</td>
<td>3011 ± 312</td>
<td>MTP</td>
<td>18 ± 3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fischer rats</td>
<td>None</td>
<td>296 ± 53</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>MTP</td>
<td>2172 ± 232</td>
<td>MTP</td>
<td>None</td>
</tr>
<tr>
<td>AKR mice</td>
<td>None</td>
<td>3318 ± 567</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>MTP</td>
<td>5429 ± 416</td>
<td>MTP</td>
<td>None</td>
</tr>
<tr>
<td>C57BL/J mice</td>
<td>None</td>
<td>3834 ± 357</td>
<td>MTP</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>MTP</td>
<td>3884 ± 485</td>
<td>MTP</td>
<td>None</td>
</tr>
<tr>
<td>BALB/c, nude</td>
<td>None</td>
<td>1942 ± 189</td>
<td>MTP</td>
<td>None</td>
</tr>
<tr>
<td>Syrian Hamster</td>
<td>None</td>
<td>573 ± 37</td>
<td>MTP</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>MTP</td>
<td>363 ± 32</td>
<td>MTP</td>
<td>None</td>
</tr>
</tbody>
</table>

a For methodology, see "Materials and Methods."

b The dose of MTP used was 2 µg/ml. Dose-response curves with Swiss mice and Fisher rat spleen lymphocytes indicated that a dose of 1 to 2 µg MTP per ml gave the maximum mitogenic response in the range 0.1 to 50 µg MTP per ml tried. The values obtained above are for 4 Fischer rats and for mixed spleen lymphocytes of 5 mice of each strain in 4 separate experiments.

c The dose of MTP used was 10 µg/ml. Dose-response experiments with 0.1, 10, and 50 µg MTP per ml indicated little response with 0.1 µg and about 30% lesser response with 50 µg than that obtained with 10 µg MTP. The values are an average for 2 animals in each experiment.

d The PFC dose response with 0.1, 10, and 50 µg MTP injected s.c. per animal gave little response with 0.1 µg and approximately the same response with 10 and 50 µg MTP. In these experiments a dose of 10 µg MTP was used. These values were obtained with 4 animals each in untreated and MTP-treated groups.

e Averages ± S.D. of 5 wells.

The antitumor activity of MTP is confined to its ability to (a) enhance host capacity to reject a larger number of tumor cells, (b) retard growth in tumor size, and (c) induce hemorrhagic necrosis in certain tumors. However, 3 other large fast-growing tumors did not regress under the influence of MTP alone, and an insignificant increase in the life span of such animals treated with MTP was obtained. Thus, MTP differed from endotoxin, which has been shown by Parr et al. (11) to cause hemorrhagic necrosis after the tumor transplant establishes vasculatization, and is without effect when injected on Day 0 after tumor implantation. However, these authors did not determine whether the viable cell inoculum for 50% tumor takes of tumor cells was enhanced on endotoxin treatment starting from Day 0. Our own unpublished experiments have shown that bacterial
glycolipid mR595 is no more effective than is MTP against growing as well as freshly transplanted MAC, although a combination of MTP and glycolipid mR595 offers a definite advantage over MTP treatment alone.

The degree of antitumor activity elicited by MTP depends on the tumor-host system used. Based on the number of inoculated tumor cells rejected by the host on MTP treatment, MAC was the least sensitive; NH, CI2TSV5S, and B16 were of intermediate sensitivity; and L26 was the most sensitive. The reason for the different degrees of sensitivity of various tumors to MTP treatment probably lies with several factors including immunogenicity of the tumor, the rate of its growth, and the ability of the tumor to release factors antichemotactic for macrophages (17).

The mode of antitumor action of MTP appears to be via its action on the immune system of the host, since the ability of MTP to reject a small number of tumor cells is destroyed by 400 rads X-irradiation of animals. By itself MTP is noncytotoxic to tumor cells in vitro, since viability of cells as seen by trypan blue exclusion is not affected by incubation of tumor cells with MTP (5 µg/ml) for 2 to 3 days. We also found that MTP mitogenically stimulated spleen lymphocytes of certain species and strains of animals, although tumor rejection occurred in both mitogenic responders and nonresponders. Further, MTP enhanced Fischer rat spleen PFC and serum antibody titers with SRBC used as antigen. However, antitumor antibody titers were enhanced only slightly on MTP treatment (Table 2, see Footnote e). Only animals that had rejected MAC or NH, in the absence or presence of MTP treatment, showed an increase in antitumor antibodies. It seems likely that tumor rejection leads to antibody appearance in the serum rather than that antibodies cause tumor destruction. However, it is possible that MTP-induced antitumor antibodies lead to specific arming of macrophages, cytotoxic to the tumor cells.

In this study we could also demonstrate that activation of Fischer rat peritoneal macrophages was obtained by in vitro MTP treatment. Furthermore, Fischer rats and hamsters transplanted with their respective tumors and then treated with MTP gave peritoneal macrophages that were much more cytotoxic to tumor cells than were those derived from animals receiving tumor cells of MTP alone. However, we have no reason to believe that MTP activates macrophages specifically against the implanted tumor. Additional work with several tumor target cells is required to show whether either a greater or a specific activation of peritoneal macrophages in tumor-transplanted animals occurs in comparison to MTP alone treated animals.

In spite of the presence of activated macrophages in the MAC-bearing MTP-treated Fischer rats, this tumor is more resistant to MTP-induced tumor regression than is CI2TSV5S tumor in hamsters. It is possible that either MAC releases factors that are antichemotactic for macrophages or this tumor is poorly immunogenic so that T-cells are insufficiently sensitized to deliver a chemotactic signal to activated macrophages.

Although the mode of action of MTP is yet unclear, our recent comparative experiments (H. El Kappany, C. Chopra, V. N. Nigam, C. A. Brailovsky, and M. Elhilali, unpublished results) with BCG and MTP against Dunning R-3327-H transplantable prostatic carcinoma in Copenhagen-Fischer F1 male hybrid rats demonstrate that MTP was more effective than was BCG when the tumor burden was small. Both were ineffective against large tumors, even when injected i.t. However, BCG (13) and BCG cell walls plus trehalose mycolate (22) have been reported to be very promising against line 10 hepatocellular carcinoma in guinea pigs.

REFERENCES
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