Phase I and Immunomodulatory Study of a Muramyl Peptide, Muramyl Tripeptide Phosphatidylethanolamine

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ABSTRACT

Muramyl tripeptide phosphatidylethanolamine (MTP-PE; CGP 19835A from Ciba Geigy) is a synthetic muramyl tripeptide structurally related to bacterial cell wall constituents. MTP-PE activates monocytes in vitro to a tumoricidal state and has in vivo antitumor effects in animal models. We studied the toxicity and immunomodulatory effects of once weekly i.v. administration of liposomal-encapsulated MTP-PE for 8 weeks in 27 patients with advanced malignancies. Doses ranged from 0.1 to 2.7 mg/m². No major tumor responses were seen; 11 patients had stable disease after 8 weeks of therapy and 3 continued on maintenance therapy because of minor tumor regressions and/or clinical improvement. MTP-PE at these doses was well tolerated. Shaking chills and fevers were the most common toxicities and occurred at all dose levels. There was no treatment-induced loss of performance status. Immunomodulatory studies revealed evidence of a biological effect on monocytes. C-reactive protein levels rose in the majority of patients with end-of-treatment values 2 to 10 times higher than baseline. Serum neopterin levels were consistently increased 24 h after MTP-PE administration and significant decreases in expression of two different types of Fc receptors on peripheral blood monocytes were noted 6 h after treatment. Although no major tumor responses were seen in this group of patients with advanced malignancies, MTP-PE was well tolerated and exerted biological effects on monocytes. Serum neopterin levels may be a useful marker for the biological effects of MTP-PE.

INTRODUCTION

Peripheral blood monocytes and tissue macrophages participate in various aspects of the immune system. They process antigen and present it to T-cells, and as effector cells, activated monocytes/macrophages can recognize and kill tumor cells (1). Activating monocytes to a tumoricidal state is one immunotherapeutic approach to the treatment of cancer. Tumoricidal monocytes are selective for neoplastic cells in vitro (2) and can kill tumor cells despite their phenotypic heterogeneity and regardless of their growth rate or susceptibility to cytotoxic T-cells or natural killer cells (3). Moreover, tumor cell variants resistant to macrophage-mediated lysis evolve uncommonly, even under selective pressure (4). There are a variety of methods to activate monocytes. Lymphokines such as IFN-γ, tumor necrosis factor (TNF) and interferon-α (IFN-α) are involved in activating monocytes/macrophages to tumoricidal activity (5). Monocytes/macrophages can recognize and kill tumor cells (1). Monocytes/macrophages can also recognize and kill tumor cells despite their phenotypic heterogeneity and regardless of their growth rate or susceptibility to cytotoxic T-cells (5).

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search Facility. All patients gave written informed consent.

Drug Supply and Preparation. The drug MTP-PE (CGP19835A) was supplied already formulated by Ciba-Geigy, Ltd., Basel, Switzerland. MTP-PE is a synthetic muramyldipeptide coupled to dipalmitylphosphatidylethanolamine. It is N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-[(1,2-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)ethylamide monosodium salt encapsulated in a multilamellar liposome consisting of synthetic phosphatidylcholine and phosphatidylserine in a 7.3 molar ratio. The lipid:MTP-PE ratio in the final preparation was 250:1. The drug was reconstituted fresh in 50 ml of endotoxin-free isotonic saline for i.v. injection.

Study Design. This was a single arm, dose-escalating phase I study that was one of multiple studies examining the toxicity and immunomodulatory properties of liposome-encapsulated MTP-PE. Our study was designed to determine the toxicity and immunomodulatory effects of once-weekly administration at the following doses: 0.1, 0.2, 0.4, 0.8, 1.2, 1.8, and 2.7 mg/m². Preliminary results from other trials had already established the safety of doses below 0.1 mg/m² so this was chosen as our starting dose. Because of our concern that previous treatment with different doses of a biological agent could affect the immunomodulatory properties of subsequent doses, we decided there would be no intrapatient dose escalation. Our dose escalation stopped at 2.7 mg/m² without determining a MTD because other studies in which intrapatient dose escalation was used had reached dose-limiting toxicity with twice-weekly administration at doses approximately 5-fold higher. MTP-PE was administered by i.v. infusion over 1 h once weekly for 8 weeks. Patients were eligible for additional treatment if they experienced clinical improvement or had a tumor response. Each patient was hospitalized for at least the first dose of MTP-PE and observed overnight. Subsequent doses could be administered in the outpatient setting. Patients were seen by a physician before and after each treatment. Clinical status and vital signs were monitored for 4 h after each treatment. Serum chemistries, complete blood count, urinalysis, PT, PTT, fibrinogen, and fibrin degradation products were checked weekly, 24 h after each treatment. Because of the preclinical evidence of vascular toxicity, EKGs were checked just before and 24 h after each dose and each physical examination included a fundoscopic examination. Antinuclear antibody, rheumatoid factor, and quantitative immunoglobulins were checked at baseline and at the completion of the study. C-reactive protein was checked pretreatment, midstudy, at week 2, 4, 6, and 8, and 1 month poststudy.

Immunological Monitoring. In the majority of patients, immunological testing was performed on serum and peripheral blood mononuclear cells obtained from patients at baseline (2-3 samples) and just before and 24 h after the first, fifth, and eighth (last) doses of MTP-PE. Whole blood was collected in preservative-free heparin and peripheral blood mononuclear cells were obtained by centrifugation through Ficoll-Hypaque gradients. Peripheral blood mononuclear cells were used fresh or suspended in RPMI with 10% fetal calf serum and 10 mM dimethyl sulfoxide (Fisher, Silver Spring, MD), frozen using a Cryo-Med controlled rate freezer (Mt. Clemens, MI), and stored in liquid nitrogen. Serum was isolated and maintained at −20°C.

Flow Cytometry. Phenotyping of all samples from an individual patient was performed on a single day to minimize variability in staining and instrument calibration. Mononuclear cells were incubated in phosphate-buffered saline containing 2% heat-inactivated human AB serum for 5 min at room temperature to block Fc receptors prior to incubation with monoclonal antibodies. Fluorescein-conjugated antibodies to HLA-DR, HLA-DQ, and CD14 (Leu-M3) and unconjugated anti-HLA-DP were obtained from Becton-Dickinson Immunocytometry Systems (Mountain View, CA). Detection of IgG Fc receptors was performed using monoclonal antibodies 32.2 (11) and IV.3 (12) which were generously supplied by Dr. Paul Guyre, Dartmouth University. The monoclonal antibody 32.2 recognizes FcRI. The epitope recognized by 32.2 is not blocked by interaction of FcRI with human IgG (13). Monoclonal antibody IV.3 recognizes FcRII. Cells incubated with unconjugated primary antibodies were washed and incubated with fluorescein-conjugated goat anti-mouse IgG (Tago, Inc., Burlingame, CA). After staining, residual RBC were lysed by incubation in buffered ammonium chloride. Propidium iodide was added to each sample at a final concentration of 1 µg/ml to allow discrimination of viable from nonviable cells.

Flow cytometric analysis of patient cells was performed on either an Ortho Cytofluorograf 30 or a Coulter EPICS Profile flow cytometer. Lymphocyte and monocyte populations were bitmap gated based on light scatter and viable, propidium iodide-negative cells within the bitmaps were analyzed. Gating effectiveness was confirmed by CD14 staining of monocytes. Histograms of log green fluorescence were collected and the percentage of positive cells was determined by comparison to isotype controls. The MFI of antibody-positive cells was also determined.

C-reactive Protein. C-reactive protein was measured using a particleenhanced turbidimetric immunoassay adapted to the DuPont ACA analyzer. This method uses a single-pack, rate technique to measure CrP. The CrP pack contains latex particles coated with a CrP antibody. CrP present in the serum causes concentration-dependent aggregation of the latex particles which is measured turbidimetrically at 340 nm. The concentration is determined by means of a previously prepared lot-specific computerized mathematical function. The normal range for this assay is a CrP value of <0.9 mg/ml.

Neopterin. Serum levels of neopterin were measured using a radioimmunoassay kit (Neopterin-RIAKit; HENNING Berlin GMBH, Berlin, Federal Republic of Germany). The manufacturer's instructions were followed in the performance of this assay. The normal value is <10 nmol/liter.

Interferon. IFN-γ was detected by a RIA obtained in kit form (IMRX Interferon-Gamma RIA; Centocor, Inc., Malvern, PA). The manufacturer's instructions were followed in performing this sandwich RIA. Specimens were incubated with polystyrene beads coated with monoclonal anti-IFN-γ antibodies. A second, 125I-labeled monoclonal antibody bound to the immobilized interferon in positive wells, and the beads were counted in a gamma counter. IFN-γ standard was provided with the kit. The sensitivity is approximately 0.1 units/ml.

Measurement of H2O2 Release by Monocytes. H2O2 production by mononuclear phagocytes was determined by monitoring the horseradish peroxidase-catalyzed oxidation of fluorescent scopoletin (14). The results were calculated by microcomputer and expressed as nmol of H2O2 produced/µg of protein/h. This method has been described in detail previously (15).

Statistical Methods. Changes from baseline for serum levels of neopterin and CrP were assessed using a paired t test; within-group and between-group comparisons were made by subtracting average baseline measurements from average on-treatment measurements for each patient and using paired and two-sample t tests, respectively (16). The statistical test results described are based on two-sided tests conducted at a significance level of 0.05.

Methods of analysis on Fc receptor response included one-way analysis of variance, two-way repeated measures analysis of variance, and profile analysis. The two-way repeated measures technique allowed for examination of an effect upon the Fc receptor number (dependent variable) as a result of possible interaction among time and dose (independent) variables. Specifically, we examined changes in doses among patients and changes across weeks and hours within a given week and their possible interaction on the Fc receptor response.

For many analyses, appropriate error structure residuals were obtained from the following repeated measures structural model:

\[ Y_{ik} = U + D_i + T_j + DT_{ij} + S_{ik} + TS_{ikj} + E_{ik} \]

where \( Y_{ik} \) is the Fc measurement on the \( k \)th patient, \( U \) is the grandmean, \( D_i \) is the effect due to the \( i \)th dose, \( T_j \) is the effect due to the \( j \)th h (or week), \( DT_{ij} \) is the effect due to interaction between dose and time, \( S_{ik} \) is the effect due to patient \( k \) within dose group \( i \), \( TS_{ikj} \) is the interaction between time (\( j \)th h or week) and patient \( k \) in dose group \( i \), and \( E_{ik} \) is an error (residual) specific to patient \( k \) which is not measur-

The structural model partitions the total variation into additive components and identifies appropriate variance estimates for testing hypotheses about dose and time effects.

Response Criteria. Standard criteria for tumor response were used. An objective response required greater than a 50% reduction in the
sum of the products of bidimensional measurements of tumor lasting for 30 days without growth of other lesions or appearance of new lesions; disease progression was defined as ≥25% increase in the sum of the products of bidimensional measurements. Any patient who met neither of these criteria was considered to have stable disease.

RESULTS

Patient Population

The characteristics of all 27 patients entered in this study are given in Table 1. Twenty-five patients are evaluable for toxicity and immunological changes; one patient each was removed after only one dose of MTP-PE because of the development of progressive disease and brain metastases, respectively. Four other patients were removed prior to completion of all 8 weeks of therapy (at 5, 6, 6, and 7 weeks) because of progressive disease requiring alternate therapy but are included in our evaluation of toxicity and immunological effects. Twenty-one patients completed all 8 weeks of therapy. The number of patients treated at each dose level is listed in Table 2. No objective responses were seen; 11 patients had stable disease after 8 weeks of therapy. Among the 11 patients with stable disease, 3 had evidence of minor tumor regressions and/or clinical improvement and were continued on therapy until they developed progressive disease after an additional 8, 8, and 10 weeks of therapy, respectively.

Toxicity. MTP-PE at the dose levels tested was generally well tolerated. Mild to moderate chills and fever were seen in most patients and at all dose levels. Patients were premedicated routinely with acetaminophen and meperidine was used as needed for chills. Several patients reported mild nausea often after meperidine administration. Grade 1 hypotension, defined as a 20 mm Hg drop in systolic blood pressure that did not require medical intervention, occurred in 12 patients. This was seen sporadically, occurring in only 21 of the 93 (23%) doses of MTP-PE given to those 12 individuals. Three other patients had transient systolic blood pressure decrements of 20–30 mm Hg that required medical intervention (grade 2). This occurred after the first dose in a patient given 1.8 mg/m² and responded promptly to fluids. Hypotension did not recur and this patient was able to work full time during the remaining 7 weeks of therapy. Both patients treated at the 2.7-mg/m² level developed asymptomatic grade 2 hypotension on two separate occasions. Fluids were administered for one of these episodes while the others resolved without intervention. Typically, a patient developed a shaking chill within 1–2 h of drug administration that was followed by a monophasic fever. Hypotension, if it occurred, followed thereafter and was often unaccompanied by tachycardia. Occasional patients noted fatigue on the day of therapy but usually recovered within 24 h of the dose, and there was no treatment-related loss of performance status. No patient required dose modification because of toxicity and there were no thromboembolic events or changes on EKG or funduscopic exams. The frequency and severity of side effects were not related to the week of treatment.

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>No. of patients entered</th>
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</tr>
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<tbody>
<tr>
<td>Male/female</td>
<td>19:8</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>57 (39–72)</td>
</tr>
<tr>
<td>Performance status (ECOG) at entry</td>
<td>0</td>
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<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Tumor types</td>
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</tr>
<tr>
<td>Colorectal</td>
<td>4</td>
</tr>
<tr>
<td>Melanoma</td>
<td>4</td>
</tr>
<tr>
<td>Renal cell</td>
<td>4</td>
</tr>
<tr>
<td>Lung (squamous)</td>
<td>1</td>
</tr>
<tr>
<td>Rectum (squamous)</td>
<td>1</td>
</tr>
<tr>
<td>Ovarian</td>
<td>1</td>
</tr>
<tr>
<td>Trachea (adenoid cystic)</td>
<td>1</td>
</tr>
<tr>
<td>Prior therapy</td>
<td>10</td>
</tr>
<tr>
<td>Irradiation</td>
<td>8</td>
</tr>
<tr>
<td>Chemotherapy only</td>
<td>12</td>
</tr>
<tr>
<td>Immunotherapy</td>
<td>11</td>
</tr>
<tr>
<td>Multiple prior therapies</td>
<td>5</td>
</tr>
<tr>
<td>None</td>
<td>5</td>
</tr>
</tbody>
</table>

*Unless otherwise noted, values represent number of patients.

Table 2 Number of patients at various dose levels

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>Entered</th>
<th>Completed 1 full cycle</th>
<th>Received maintenance therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>0.2</td>
<td>5</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>0.4</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>0.8</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>1.2</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>1.8</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>2.7</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Hematological Effects

There were no significant dose-related changes in total WBC, granulocyte, monocyte, or lymphocyte counts in patients during treatment with MTP-PE. Similarly, there were no significant effects on hemoglobin, hematocrit, or platelet counts.

Immunological Monitoring

Neopterin. Most patients receiving MTP-PE doses of 0.2 mg/m² or higher had serum samples obtained before and after the first, fifth, and eighth doses (days 0, 1, 28, 29, 49, and 50) for determination of neopterin levels. Neopterin levels were increased 24 h after each dose of MTP-PE (Fig. 1). The maximal increase in neopterin was seen after the first dose; the 98% increase after the first dose was significantly greater than the 51 and 49% increases seen after the fifth and eighth doses, respectively. The increases observed after the fifth and eighth doses, however, were significantly different from baseline (P < 0.0005 and P < 0.01, respectively). There did not appear to be chronic elevation of neopterin levels since in patients who were examined weekly, the serum neopterin level generally returned...
to normal by the next dose 1 week later (data not shown). The neopterin levels just before the first (9.8 nmol/liter), fifth (11.0 nmol/liter) and eighth (13.5 nmol/liter) doses, although showing an increasing trend, were not significantly different from each other (Table 3). The increases in serum levels of neopterin were observed at all doses of MTP-PE. Higher doses of MTP-PE did not induce higher levels of neopterin, although the small number of patients treated at each dose make this difficult to determine reliably.

γ-Interferon. Serum was examined for secondary induction of γ-interferon in patients at all doses except 0.1 mg/m². No measurable IFN-γ was found in patients treated at any of the dose levels.

C-reactive Protein. Serum levels of C-react were examined at baseline, 24 h after the fifth (day 29) and eighth (day 50) doses of MTP-PE, and approximately 1 month after completion of the study. C-react levels rose in the majority of patients during treatment. Increases were usually modest, ranging from a 2- to 10-fold rise. When all patients were combined, C-react levels were significantly increased on day 29 (P < 0.0007) and day 50 (P < 0.031) (Table 4). Increases in C-react did not appear to be dose related since the percentage increase above baseline was similar regardless of the MTP-PE dose. C-react levels had returned to pretreatment values within 1 month of completing therapy.

Monocyte Cell Surface Markers

Fc Receptor Expression. Expression of the high affinity (FcRI) and low affinity (FcRII) Fc receptors on peripheral blood monocytes was examined at 6 and 24 h following the first, fifth, and eighth doses of MTP-PE. MTP-PE treatment did not change the percentage of monocytes bearing either type of FcR; virtually all monocytes were positive for both FcRI and FcRII throughout treatment. FcRI was measured directly using monoclonal antibody 32.2 and FcRII was measured using the IV.3 antibody.

The repeated measures analysis-of-variance model described earlier permitted the partitioning of the total variance in the response measure (fluorescence intensity) into components which corresponded to three hypotheses of interest, namely: (a) possible time effects; (b) possible dose effects; and (c) possible interactions between dose and time. The findings were similar for FcRI and FcRII and will be discussed together. There were no effects due to dose nor were there significant interactions between dose and time. Also, there were no time effects across

weeks at a particular hour. For example, at 0 h mean responses were statistically equal in the first, fifth, and eighth weeks of the study. The same was also true at 6 and 24. As indicated in Table 5, however, there were consistent and significant decreases in mean fluorescent intensity 6 h following MTP-PE administration. At 24 h, the fluorescent intensity measurements returned to baseline. Since light scatter analysis failed to detect any change in the size of monocytes, we attribute the decreased fluorescence to a decrease in the number of cell surface FcR.

Table 4  C-reactive protein

<table>
<thead>
<tr>
<th>Day</th>
<th>N</th>
<th>Mean ± SE (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>17</td>
<td>3.5 ± 0.9</td>
</tr>
<tr>
<td>29</td>
<td></td>
<td>6.9 ± 0.9</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>5.5 ± 1.0</td>
</tr>
</tbody>
</table>

a Serum neopterin levels were measured as described in "Patients and Methods." Units are nmol/liter.

b Values before each dose not significantly different from each other.

c Significantly different from percentage increases on days 29 and 50.

Table 5 Monocyte Fc receptor expression

<table>
<thead>
<tr>
<th>Nα</th>
<th>ΔMFCβ</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FcRI</td>
<td>6 h</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>48</td>
</tr>
<tr>
<td>FcRII</td>
<td>6 h</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>43</td>
</tr>
</tbody>
</table>

α N, number of patient samples; NS, not significant; ΔMFC, change from baseline in mean fluorescent channel; positive number indicates decrease in Fc receptor expression; positive number indicates increase in Fc receptor expression.

Fig. 2 demonstrates these results at week 8 for FcRI and FcRII, respectively. Similar results were obtained at weeks 1 and 5 indicating that the same pattern was consistent across weeks. In Fig. 24 FcRI means for dose groups 0.1, 0.2, 0.4, and 1.8 are plotted at 0, 6, and 24 h following MTP-PE administration. Fig. 24 demonstrates that there is no upward or downward trend among dose groups. However, there is a significant drop at all doses of MTP-PE 6 h after administration. The lack of significant dose by time interaction is indicated by the parallelism of mean responses among dose groups.

Fig. 2B shows a similar pattern for FcRII receptor means; however, the drop 6 h following administration is even more dramatic. The FcRII data are statistically parallel and, again, show no trend in means among dose groups.

Occasional patients experienced an increase in FcRI expression as illustrated in Fig. 3. Patient 25 experienced an increase in FcRI expression and a decrease in FcRII expression after his first, fifth, and eighth doses of MTP-PE at 1.8 mg/m². The increase in FcRI expression was generally seen within 6 h of drug administration and persisted for 24 h whereas FcRII expression decreased dramatically at 6 h but returned to baseline within 24 h.

Class II MHC Antigen Expression. For most patients, the percentage of positivity or MFI of HLA-DR, -DQ, or -DP did not change substantially. More than 90% of monocytes routinely stained positive for HLA-DR at baseline and this was true throughout the study period. There were no consistent dose-related changes in percentage positivity or MFI observed during the study; however, occasional patients exhibited increases in expression of class II MHC antigens following MTP-PE administration. Typical changes in Class II antigen expression are shown for patient 4 in Fig. 4. After receiving his fifth 0.2-mg/m² dose of MTP-PE, peripheral blood monocytes were shown to have enhanced expression of HLA-DR, -DQ, and -DP antigens. In addition, the percentage of monocytes positive for HLA-DQ and -DP were also increased.

CD14 Expression. When all doses of MTP-PE are considered, there was no consistent dose-related effect of MTP-PE on monocyte expression of CD14. However, there was a trend toward decreased CD14 expression as early as 6 h after drug administration which persisted for as long as 24 h. Certain patients exhibited dramatic changes in CD14 expression. The change in CD14 expression in patient 4 is depicted in Fig. 3. One can see a marked decrease in mean fluorescent channel
PHASE I AND IMMUNOMODULATORY STUDY OF MTP-PE

Fig. 2. Change in MFI of cells in the monocyte light scatter window after staining for FcRI (32.2) and FcRII (IV.3). The changes occurring during the 24 h following the eighth and last dose of MTP-PE were chosen. The means presented at each dose reflect only those patients treated at each dose for whom complete data were available. The doses are in mg/m². A. FcRI; B. FcRII.

Fig. 3. Change compared to baseline of the high affinity (FcRI) and low affinity (FcRII) Fc receptor after the fifth and eighth doses of MTP-PE at 1.8 mg/m² in patient 25. Baseline was calculated by averaging the mean fluorescent channel of three pretreatment samples stained with fluorescein isothiocyanate-labeled FcRI and FcRII, respectively. The data depicted were from 24 h after MTP-PE for FcRI (•) and 6 h after MTP-PE for FcRII (□).

Fig. 4. Change in monocyte expression of the cell surface markers HLA-DR, HLA-DQ, HLA-DP, and CD14 before (day 28) and after (day 29) the fifth dose of 0.4 mg/m² of MTP-PE in patient 4 is depicted. Cells were stained with fluorescein isothiocyanate-labeled antibodies and the percent of positive (POS) cells and mean fluorescent channel were calculated as described in “Patients and Methods.”

Lymphocyte Cell Surface Markers

There were no significant changes in the percentage of positive cells or mean fluorescent intensity of cells bearing the CD3, CD4, CD8, CD19, or Leu-19 antigens.

Functional Assays

Attempts were made to assess the cytotoxicity of monocytes from MTP-PE-treated patients by measuring their ability to kill A375 melanoma cells in a 72-h in vitro assay (17). Unfortunately, the variability of this assay was too great to permit a reliable assessment of cytotoxicity. As a surrogate measure of monocyte activation, we examined phorbol myristate acetate-induced hydrogen peroxide (H₂O₂) production in monocytes. No significant dose-related effects were observed. When all MTP-PE dose levels were combined and H₂O₂ production at 24 h following the first, fifth, and eighth doses was examined, the only significant change was a modest decrease in H₂O₂ production 24 h after the eighth dose (P < 0.039).

DISCUSSION

We performed a phase I study of liposomal-encapsulated MTP-PE in patients with advanced malignancies. Our original objectives were to determine both a maximally tolerated dose (MTD) and an immunologically active dose of MTP-PE when given once weekly. Weekly outpatient i.v. administration of liposomal-encapsulated MTP-PE at doses from 0.1 to 2.7 mg/m² was safe and well tolerated. The toxicity was mild (primarily fever and chills) and similar to other biological agents. Our dose escalation was stopped before a MTD was determined because other investigators using twice-weekly administration with intrapatient dose escalation had determined the MTD to be 6 mg/m² (18). No objective tumor regressions were seen in our cohort of patients or in those of the other published phase I study (18).

Although we were unable to determine an optimal immunomodulatory dose, our data suggest that MTP-PE results in a myriad of biological effects on monocytes. We observed in-

from 502 to 332 and the presence of a number of CD14- monocytes since the percentage of cells bearing the CD14 antigen decreased from 87 to 79.
cytes/macrophages are the primary source of neopterin. Neopterin production accompanies ongoing immune responses (22). Whether MTP-PE caused CrP production directly or indirectly via induction of other cytokines cannot be determined from our data.

The consistent increase in serum neopterin levels after MTP-PE administration is, to our knowledge, a new finding. Neopterin is a pteridine compound derived from GTP (23, 24). Although no effector function has been ascribed to this molecule, neopterin production accompanies ongoing immune responses (25). Huber et al. (25) have demonstrated that monocytes/macrophages are the primary source of neopterin. Neopterin production is thought to be due to the direct stimulation by IFN of GTP cyclohydrolase I which catalyzes the breakdown of GTP to dihydronopterin triphosphate (26). Since macrophages lack the next enzyme in the biosynthetic pathway to tetrahydrobiopterin synthesis, there is a buildup and release of dihydronopterin and neopterin that can be measured in serum and eventually in urine. Neopterin levels have been used to monitor allograft recipients (27) and to monitor the activity of certain autoimmune diseases (28). Others have shown elevated urinary levels of neopterin in patients treated with interleukin 2 and IFN-γ (29). Unfortunately, we could not correlate changes in neopterin with the dose of MTP-PE or with effects on monocyte function. The lack of dose-related effects suggests that doses as low as 0.1 mg/m² may already be above the maximal immunomodulatory dose. Lower doses of MTP-PE would have to be tested to determine a dose-response curve. It is also possible that administration of empty liposomes could induce monocytes to secrete neopterin regardless of their MTP-PE content; this hypothesis is being tested in vitro.

The mechanism of neopterin induction in our patients is not known. γ-Interferon is a potent neopterin inducer, but lipopolysaccharide has also been shown to directly induce monocytes to produce neopterin (26). Recent in vitro studies suggest that MTP-PE is unable to induce monocytes to produce neopterin. This may be related to the absence of secondary signals in vitro that may be present in vivo. It is unlikely that low levels of LPS contaminated the MTP-PE preparation and induced neopterin production in vivo because the MTP-PE was carefully tested for this before incorporation into liposomes. It is possible, however, that despite our inability to detect IFN-γ in patients’ sera, there may have been induction of interferon in vivo following MTP-PE administration. Goldstein et al. (30) have made similar observations with IFN-γ; despite the absence of IFN in the serum, a majority of their patients exhibited a number of biological changes associated with IFN.

IFN-γ production in our patients can be inferred from changes observed in other immune parameters. Certain patients exhibited coordinate increases in FCRI and class II MHC antigen expression (HLA-DR, -DP, and -DQ) with an accompanying decrease in monocyte CD14 expression, a finding described after stimulation of monocytes with IFN-γ in vitro (31) and also seen in our patients receiving IFN-γ. Vermeulen et al. (32) have shown that muramyl dipeptide can enhance mRNA expression for class II MHC antigens, but this is probably not sufficient to result in enhanced expression of the protein on the cell surface (33). Similarly, MTP-PE does not cause down regulation of CD14 on monocytes in vitro (33). These observations and the changes in neopterin suggest that MTP-PE administration may induce levels of IFN-γ sufficient to induce monocytes to produce neopterin but too low, except in an occasional patient, to enhance FCRI and class II MHC antigen expression or to down-regulate CD14 expression.

All the observed changes are not due simply to secondary induction of IFN-γ since the decreases in FCRI and FCRII expression seen 6 h after MTP-PE administration are not seen following IFN-γ treatment. They may be direct effects of MTP-PE or possibly may be liposome induced.

We also examined whether direct monocyte cytotoxicity against the A375 melanoma cell line (17) was affected by MTP-PE. In contrast to the results of others (34), who found activation of monocyte-mediated tumoricidal activity in a majority of their patients at some time during treatment, we observed significant variability in baseline monocyte cytotoxicity accompanied by increases in cytotoxicity after some doses and decreases in cytotoxicity after others within the same patient. The inability to document monocyte activation may be related to dose or schedule of MTP-PE administration, or it is conceivable that upon activation, monocytes left the circulation and differentiated into tissue macrophages. Similarly, local production of IFN-γ may have activated monocytes in situ but not in the peripheral blood.

The main reason to measure the immune effects of MTP-PE is to determine an optimal biological dose, which may be far different from the MTD, for phase II testing. This is important because animal data suggest that the antitumor effects of MTP-PE may be related to its capacity to activate host monocytes. The evidence for the role that macrophages play in the destruction of metastases includes: (a) macrophages activated in vitro by liposome-encapsulated MDP and injected i.v. significantly inhibit the growth of lung metastases (35); (b) when MDP is delivered in insufficient doses or in liposomes that are not sufficiently retained in the lung, little or no activation of lung macrophages was observed and growth of metastases was unaltered (35, 36); (c) doses of MDP that result in regression of established metastases are associated with tumoricidal macrophages residing in the tumor (36); and (d) antitumor effects are not observed in tumor-bearing animals treated with agents that impair macrophage function prior to treatment with liposome-encapsulated muramyl peptides (35). In an adjuvant study of osteosarcoma in dogs, twice-weekly i.v. injection of 2 mg/m² of liposome-encapsulated MDP resulted in a 20% reduction of tumor size. In contrast, treatment with i.m. liposome-encapsulated MDP or liposome-encapsulated muramyl peptides resulted in no reduction of tumor size.

Because it is possible that MTP-PE may alter host immunity, the safety and efficacy of this agent should be assessed in patients with a variety of malignancies. The principal signal for the secretion of CrP, and other acute phase proteins, is β2-interferon/hepatocyte-stimulating factor/interleukin 6, released from monocytes (or fibroblasts) after the appropriate activating stimulus (20). Endotoxin, interleukin 1, and tumor necrosis factor have been shown to enhance the secretion of interleukin 6 by monocytes (20). The recognized functions of CrP include complement activation and opsonization (19) as well as the ability to feedback positively on monocyte function. The lack of dose-related effects suggests that MTP-PE administration is, to our knowledge, a new finding. Neopterin is a pteridine compound derived from GTP (23, 24). Although no effector function has been ascribed to this molecule, neopterin production accompanies ongoing immune responses (25). Huber et al. (25) have demonstrated that monocytes/macrophages are the primary source of neopterin. Neopterin production is thought to be due to the direct stimulation by IFN of GTP cyclohydrolase I which catalyzes the breakdown of GTP to dihydronopterin triphosphate (26). Since macrophages lack the next enzyme in the biosynthetic pathway to tetrahydrobiopterin synthesis, there is a buildup and release of dihydronopterin and neopterin that can be measured in serum and eventually in urine. Neopterin levels have been used to monitor allograft recipients (27) and to monitor the activity of certain autoimmune diseases (28). Others have shown elevated urinary levels of neopterin in patients treated with interleukin 2 and IFN-γ (29). Unfortunately, we could not correlate changes in neopterin with the dose of MTP-PE or with effects on monocyte function. The lack of dose-related effects suggests that doses as low as 0.1 mg/m² may already be above the maximal immunomodulatory dose. Lower doses of MTP-PE would have to be tested to determine a dose-response curve. It is also possible that administration of empty liposomes could induce monocytes to secrete neopterin regardless of their MTP-PE content; this hypothesis is being tested in vitro.

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W. C. Kopp, unpublished observations.
liposome-encapsulated MTP-PE led to enhanced survival compared to dogs treated with liposomes alone (37). This dose of MTP-PE was well tolerated and appeared to be below the MTD. The mechanism for the antitumor effects is unknown since no direct or indirect measurements of monocyte activation were undertaken. Although there appears to be an association between macrophage activation and tumor regression, a careful dose response analysis comparing the efficacy of immunomodulatory doses with nonimmunomodulatory doses is missing. However, it would seem desirable to perform clinical trials in cancer patients with doses capable of activating monocytes. Kleinerman et al. (34) suggest that optimal biological effects are observed with doses of MTP-PE from 0.5–2.0 mg/m² while maximally tolerated doses are 4–6 mg/m². This determination was based on a macrophage cytotoxicity assay with inherent variability that has been difficult to replicate. In addition, this only tells us about circulating monocytes and not monocytes that may have left the circulation after activation. Therein lies the potential value of neopterin as an indirect measure of the state of activation of the body’s monocytes. The changes in neopterin suggest that serum levels of this marker may be a valuable indicator of the direct or indirect biological effects of MTP-PE in cancer patients. The most reliable and easiest method to determine an optimal immunomodulatory dose may depend on the products released by activated monocytes and macrophages. It is possible that while MTP-PE may provide one signal required for activation, a second signal known to be necessary for the optimal activation of monocytes to a tumoricidal state (38) may be absent. Some et al. (38) found that γ-interferon alone or nor-muramyl dipeptide alone were insufficient to stimulate monocyte cytotoxicity. In contrast, various combinations of the two agents generated significant monocyte cytotoxicity. Synergistic in vitro monocyte tumoricidal activity (39) has been observed with the combination of γ-interferon and muramyl peptide, as well as enhanced in vivo efficacy (40). In this study, although we determined an immunologically active dose when MTP-PE is given i.v. once weekly, we did not determine either a MTD or an optimal immunomodulatory dose. Given the evidence that optimal monocyte activation may require two signals, MTP-PE may be best used in combination with other agents. To this end, a pilot study with the combination of MTP-PE at varying doses with γ-interferon at an immunologically effective dose would appear to be indicated.

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