Chemotherapeutic agents have been shown to enhance the antitumor activity of biological response modifiers and cytokines in rodents and humans. The purpose of this study was 2-fold: (a) to determine whether doxorubicin (DOX) would enhance or interfere with the effect of muramyl dipeptide and lipopolysaccharide on canine monocyte activation as measured by an in vitro WEHI-164 cell cytotoxicity assay; and (b) to evaluate the in vivo effect of DOX alone and combined with liposome-encapsulated muramyl tripeptide-phosphatidylethanolamine (L-MTP-PE) on monocyte activation and serum tumor necrosis factor activity. The in vivo results showed that increasing concentrations of DOX for either 1 or 24 h incubation did not directly enhance or inhibit spontaneous or activated monocyte supernatant-mediated cytotoxicity. The in vivo study showed that monocyte supernatant-mediated cytotoxicity was increased on day 3 and significantly elevated on day 7 (P = 0.016) post-DOX (30 mg/m², single injection) administration. When DOX was given in combination with L-MTP-PE (2 mg/m², twice weekly for 3 weeks), monocyte-mediated cytotoxicity was enhanced on days 3 through 10 with a significant increase on day 10 (P < 0.001). In vivo monocyte supernatant-mediated cytotoxicity was significantly elevated in dogs receiving L-MTP-PE alone at 2 h after day 0, 7, and 14 treatment, and this response was further enhanced by DOX. Serum tumor necrosis factor activity at 2 h post-L-MTP-PE was enhanced and sustained for a longer period of time in dogs that also received DOX. We conclude that DOX administered with L-MTP-PE will enhance monocyte activation induced by DOX or L-MTP-PE alone, and suggest that DOX may be combined with L-MTP-PE early in the treatment of cancer patients.

INTRODUCTION

Monocyte/macrophage-mediated tumor cell lysis is one of the major mechanisms of the host’s defense against cancer (1). Activated macrophages can preferentially inhibit the proliferation of or destroy a wide range of syngeneic, allogeneic, and xenogeneic tumor cells without damage to normal cells by secreting a number of distinct cytotoxic substances (1, 2). Biological response modifiers such as bacteria and their products, or cytokines either alone or in different combinations are capable of activating monocytes and macrophages to become tumoricidal (3–5). MDP, a synthetic molecule that resembles the minimal structural unit of the peptidoglycan cell wall of Mycobacterium and other bacteria, and LPS are potent monocyte activators (6, 7). It has been shown in humans and rodents that MDP and a lipophilic derivative of MDP, known as MTP-PE, can activate monocytes and macrophages in vitro to kill tumor targets (8–10). In a previous unpublished study, we found that supernatants from canine adherent mononuclear cells activated by MDP had cytostatic activity against canine osteosarcoma cells. In a recent study in our laboratory, we demonstrated that MDP alone and MDP plus LPS can stimulate canine blood monocytes to secrete TNF in vitro (11). Both MDP and MTP-PE can be easily encapsulated in liposomes for systemic administration and delivery to the mononuclear phagocyte system. Recently, we have shown that L-MTP-PE is effective in delaying or preventing metastasis in dogs with spontaneous osteosarcoma in a randomized clinical trial (12–14). Furthermore, L-MTP-PE will induce TNF release in vivo in normal dogs (11).

Selected chemotherapy agents not only have significant direct antitumor activities, but also have been shown to augment the host’s immune functions (15). DOX is a potent antitumor agent, having direct cytotoxic effects on a variety of clinical and experimental tumors in mice, canines, and humans (16–18). Moreover, DOX has been shown to augment monocyte/macrophage-mediated cytotoxicity and natural killer cell activity in humans and mice (19–29). The rationale for the combination of DOX and L-MTP-PE is that DOX, which can directly kill tumor cells and may also enhance host immunity, may act synergistically or additively with L-MTP-PE to prevent the tumor metastasis and prolong survival. The purpose of this study was 2-fold: (a) to determine whether DOX would enhance or interfere with the effect of MDP and LPS on canine monocyte activation as measured by an in vitro WEHI-164 cell cytotoxicity assay; and (b) to evaluate the in vivo effect of DOX alone and combined with L-MTP-PE on TNF activity. Our hypothesis is that DOX combined with L-MTP-PE will enhance monocyte supernatant-mediated cytotoxicity and serum TNF activity when compared to either treatment alone.
phosphocholine at a 7:3 M ratio. Before administration, 40 ml of Ca²⁺- and Mg²⁺-free phosphate-buffered saline was added to vials of lyophilized lipid and 4 mg of MTP-PE. The MTP-PE:phospholipid ratio was 1:250 (mg:mg). The L-MTP-PE solution at a dose of 2 mg/m² (optimal biological dose) was kindly provided by Jeff Adams and Dr. Charles Czuprynski (University of Wisconsin, Madison, WI). These tumor cells are TNF-sensitive, and IL-1 and interferon-α- and γ-resistant (3). The WEHI-164 cell line was maintained in plastic tissue culture flasks in DMEM supplemented with 5% FBS, 20 mM N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid, 2 mM L-glutamine, 100 units of penicillin G, 100 μg streptomycin, and 0.125 μg Fungizone/100 ml DMEM, at 37°C in a humidified atmosphere containing 5% CO₂.

Isolation and Stimulation of Monocytes. Peripheral blood was collected from donor dogs in heparin (1000 units/ml blood) as an anticoagulant. The blood was diluted with half its volume with HBSS in a 50:ml centrifuge tube, inverted to mix, and 8 ml of diluted blood were layered over 4 ml of Histopaque in 15-ml centrifuge tubes. Tubes were centrifuged at 1500 × g for 10 min at room temperature. The distinct band of cells that formed between the Histopaque layer and plasma was harvested with a Pasteur pipette into another 15-ml tube, and washed in HBSS at 275 × g for 10 min. To eliminate platelet contamination, cells were washed twice in HBSS at 130 × g for 15 min. The cell pellet was resuspended in MEM supplemented with 2.5% FBS. The total number of mononuclear cells was counted on a hemocytometer, a cell smear was stained with Wright-Giemsa stain, and differential cell counts were performed by morphology to determine the percent monocytes.

The cell suspension was adjusted to a concentration of 5 × 10⁵ monocytes/ml. To each well of a 96-well flat-bottomed microtiter plate (Costar Corporation, Cambridge, MA), 0.2 ml (1 × 10⁶ monocytes) was added and cells were allowed to adhere for 90 min at 39°C and 5% CO₂. After incubation, the nonadherent mononuclear cells were removed by washing the plate twice with MEM. Remaining adherent cells were incubated for 18 h with 0.2 ml of DMEM medium alone or DMEM medium containing MPD (25 μg/ml) or MPD plus LPS (25 ng/ml). After an 18-h incubation, supernatants containing the cytotoxic moiety were collected and pooled by test group and were tested immediately, or stored at −70°C for later testing in the WEHI-164 cell cytotoxicity assay.

Experiment 1: In Vitro Study—Effect of DOX on Monocytes. Mononuclear cells were isolated and allowed to adhere as described above. After nonadherent cells were removed, remaining adherent cells were incubated for 24 h with medium alone or medium containing serial concentrations of DOX (0.5 to 500 ng/ml) for 1 and 24 h in a microtiter plate. These concentrations of DOX are comparable to plasma levels used in dog studies, i.e., the concentration of DOX in plasma 1 h and 24 h postinjection ranged from 300 to 500 ng/ml and 10 to 100 ng/ml, respectively (33, 34). For the 1-h treatment group, monocytes were cultured alone for 24 h. These 24-h treatment group, monocytes were incubated with DOX for all 24 h. After incubation, the plate was washed twice with MEM, and adherent mononuclear cells were fed with medium alone or medium containing MDP (25 μg/ml) or MDP plus LPS (25 ng/ml). After an 18-h incubation, supernatants were collected and pooled by test group and were tested immediately, or stored at −70°C for later testing in the WEHI-164 cell cytotoxicity assay.

Experiment 2: In Vivo Studies—DOX Alone. Four dogs were treated with DOX and served as untreated controls. Treated dogs received a standard (therapeutic) dose of DOX at 30 mg/kg i.v., and the control dogs were given saline. Heparinized blood was collected before initiation of treatment and at days 1, 3, 7, 10, 14, and 17 post-DOX or saline injection for assessment of in vivo monocyte cytotoxic activity and in vitro activation of monocytes with MDP/LPS. WBC counts and differentials were also performed at the indicated days on both groups of dogs. Serum was collected for assessment of TNF activity at 0, 0.5, 1, 2, 3, and 4 h on day 1 post-DOX or saline injection.

Experiment 3: In Vivo Study—DOX Combined with L-MTP-PE. Two months were allowed to lapse before starting experiment 3. The 4 dogs previously exposed to DOX in experiment 2 were given a standard (therapeutic) dose of DOX at 30 mg/kg i.v. and started on L-MTP-PE at 2 mg/kg i.v. twice weekly for 3 weeks. The 4 control dogs previously exposed to only saline in experiment 2 received L-MTP-PE at 2 mg/kg i.v. twice weekly for 3 weeks without DOX. Heparinized blood was collected before initiation of treatment and at days 1, 3, 7, 10, 14, and 17 post-L-MTP-PE treatment. Serum TNF activity was also measured at 0, 0.5, 1, 2, 3, and 4 h on days 1, 3, 7, 10, and 14 post-L-MTP-PE treatment. In addition, serum TNF activity was also measured at 0, 0.5, 1, 2, and 3 h after each L-MTP-PE injection.

To confirm that the cytotoxic factor was indeed TNF-α, a neutralization experiment was done in which serum (diluted 1:20) was added to triplicate wells that contained increasing amounts of a mAb (B154.2.1) that neutralizes rHuTNF-α. Control wells contained an irrelevant mAb (5G62).

Statistical Analysis. The significance of differences between test groups within an assay and between the various treatment groups of dogs were analyzed by the Student's t test. The significance of differences between pre- and posttreatment were analyzed using the Student's paired t test.

RESULTS

Experiment 1

In Vitro Effect of DOX on Monocyte-mediated Cytotoxicity. Combining the results from 4 dogs, we found that when monocytes exposed to all concentrations of DOX for either 1 or 24 h were compared with monocytes exposed to medium alone (control), there was no significant difference in the cytotoxic moiety released into the supernatants of the cultured monocytes.

Monocytes not exposed to DOX showed 64 ± 4% (SEM) cytotoxicity induced by MDP/LPS. The monocytes incubated with 4 concentrations of DOX for 1 h and then exposed to MDP/LPS could be activated to similar levels of cytotoxicity (50–61%) (Fig. 1). Except

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were not observed with any concentration of DOX tested (data not shown) (49%). (Fig. 2)

Monocytes incubated with increasing concentrations of DOX (0.5-500 ng/ml) alone. Bars on the right, monocytes incubated with increasing concentrations of DOX, then MDP/LPS. There was no effect of DOX on spontaneous monocyte supernatant-mediated cytotoxicity, or on the ability of monocytes to be activated by MDP/LPS (n = 4).

Fig. 1. Effect of 1-h incubation with DOX on monocyte supernatant-mediated cytotoxicity. Results shown are mean cytotoxicity ± SEM. Bars on the left, monocytes incubated with increasing concentrations of DOX (0.5-500 ng/ml) alone. Bars on the right, monocytes incubated with increasing concentrations of DOX, then MDP/LPS. There was no effect of DOX on spontaneous monocyte supernatant-mediated cytotoxicity, or on the ability of monocytes to be activated by MDP/LPS (n = 4).

Concentration of doxorubicin (ng/ml)

% Cytotoxicity

Medium alone
DOX x 1 hr
MDP +LPS
DOX x 1 hr, then MDP/LPS

0 0.5 5 50 500

Fig. 2. Effect of 24-h incubation with DOX on monocyte supernatant-mediated cytotoxicity. Results shown are mean cytotoxicity ± SEM. Bars on the left, monocytes incubated with increasing concentrations of DOX (0.5-500 ng/ml) alone. Bars on the right, monocytes incubated with increasing concentrations of DOX, then MDP/LPS. There was no effect of DOX on spontaneous monocyte supernatant-mediated cytotoxicity, or on the ability of monocytes to be activated by MDP/LPS (n = 4).

Concentration of doxorubicin (ng/ml)

% Cytotoxicity

Medium alone
DOX x 24hr
MDP+LPS
DOX x 24hr, then MDP/LPS

0 0.5 5 50 500

for the highest concentration of DOX (500 ng/ml), monocytes incubated with the other 3 concentrations of DOX for 24 h could be activated by MDP/LPS to the same level of cytotoxicity (53-56%) as monocytes not exposed to DOX. Although MDP/LPS could stimulate monocytes incubated with 500 ng/ml DOX for 24 h, cytotoxicity (27 ± 21%) was decreased, although it was not statistically different (P = 0.067) compared with that of monocytes exposed only to MDP/LPS (49%). (Fig. 2)

To determine whether trace amounts of DOX may have been in supernatants and were cytotoxic to target cells, the 4 concentrations (0.5, 5, 50, and 500 ng/ml) of DOX were tested for direct effects on the WEHI-164 cells. Cytotoxic effects against the WEHI-164 cell line were not observed with any concentration of DOX tested (data not shown). In previous studies, we have shown that MDP (25 µg/ml), LPS (25 ng/ml), and MDP/LPS had no direct cytotoxic effect on WEHI-164 cells (data not shown).

Experiment 2

Effect of DOX on in Vivo Monocyte-Supernatant-mediated Cytotoxicity and on in Vitro Monocyte Activation by MDP/LPS. Supernatants from cultured monocytes before administration of DOX or saline were cytotoxic at 10 ± 3.5% and 11 ± 3%, respectively. When DOX (30 mg/m²) alone was administered, supernatant cytotoxicity was increased at day 3 (34 ± 5%) and significantly elevated at day 7 (42 ± 7.5%; P = 0.016) after administration (Fig. 3). Monocyte supernatant cytotoxicity from control dogs (saline treated) showed no change throughout the study (Fig. 3). Monocytes harvested from all dogs before and days 1, 3, 7, 10, 14, and 17 post-DOX treatment could be activated in vitro by MDP/LPS to the same levels as that of control (saline)-treated dogs (data not shown). We found no evidence that a single i.v. injection of DOX induced the secretion of TNF in the serum (data not shown).

Effect of DOX on the Number of WBCs and Monocytes. The mean number of circulating WBCs before administration of DOX was 11,600 ± 3,546/μl. This number was slightly decreased but still within the normal range (5,000 to 17,000/μl) between day 3 and day 10 posttreatment (7,075 and 6,400/μl, respectively). The WBCs had returned to pretreatment numbers by day 14. The number of circulating monocytes decreased to the lowest level at day 3 (79 ± 13/μl) post-DOX treatment and returned to pretreatment counts by day 10 (922 ± 243/μl) (Table 1). These data indicate that a single i.v. injection of DOX can transiently reduce the number of WBCs and monocytes. The control dogs had no difference in the number of total WBCs or monocytes before and after treatment (data not shown).

Experiment 3

Effect of L-MTP-PE and DOX on in Vivo Monocyte-Supernatant-mediated Cytotoxicity. Dogs treated with saline followed by L-MTP-PE twice weekly for 3 weeks showed no increase in in vivo monocyte supernatant cytotoxic activity at days 1, 3, 7, 10, 14, and 17 postsaline injection and immediately before each L-MTP-PE treatment (Fig. 3), although monocytes collected each time could be activated in vitro by MDP/LPS (data not shown). However, we found that in vivo monocyte supernatant cytotoxicity was significantly increased in dogs at 2 h after each L-MTP-PE treatment was administered (Fig. 4). When dogs were given injections of DOX followed by
L-MTP-PE, their in vivo monocyte supernatant cytotoxicity at 2 h after each L-MTP-PE treatment was further enhanced compared with that of dogs receiving L-MTP-PE alone, in which only at treatment 3 (day 7) was the increase significant ($P < 0.005$, Fig. 4). In the second experiment, we showed that in vivo monocyte-supernatant cytotoxicity was increased at day 3 (34 ± 5%) and significantly elevated at day 7 (42 ± 7.5%; $P = 0.016$) after dogs were administered DOX alone. In this experiment, we found that, when dogs received DOX combined with L-MTP-PE, monocyte supernatant cytotoxicity activity on days 3, 7, and 10 was further enhanced when compared to those dogs receiving either DOX alone or L-MTP-PE alone. This enhancement was statistically significant at day 10 ($P < 0.005$) (Fig. 3).

**Effect of L-MTP-PE and DOX on Serum TNF Activity.** We found that serum TNF activity rapidly increased within 2 to 3 h after the first L-MTP-PE alone injection and subsequently declined to pretreatment level at 4 h postadministration. No TNF activity was measured at 24 or 48 h postinjection. However, repeated i.v. injections of L-MTP-PE alone for 5 times (twice weekly) after the first injection failed to show peak levels of TNF activity at 2 h postadministration (Fig. 5). When dogs received L-MTP-PE combined with DOX, the same pattern of serum TNF activity was found after the first treatment as described for those dogs given L-MTP-PE alone (Fig. 5). However, evaluation of serum TNF activity in dogs that received DOX showed that the level of TNF produced at 2 h after each L-MTP-PE treatment was enhanced, and such response was seen after all subsequent L-MTP-PE injections (Fig. 5).

**Neutralizing Antibody to rHuTNF-α Abrogates the Cytotoxic Activity of Canine Serum.** A mouse anti-rHuTNF-α mAb was used to demonstrate that the serum cytotoxic activity was indeed caused by TNF. The anti-rHuTNF-α mAb significantly reduced the cytotoxic activity of the serum samples (1:20 dilution) collected at 2 h after the first injection of L-MTP-PE combined with DOX (Fig. 6). The effectiveness of this mAb was verified by showing that 50 μg of mAb completely neutralized the cytotoxic activity of 100 pg rHuTNF-α (95 ± 2% cytotoxicity for 100 pg rHuTNF-α versus 6 ± 7% cytotoxicity for 100 pg rHuTNF-α plus 50 μg of mAb). The same isotype of irrelevant mAb used as control had no effect on cytotoxicity induced by positive sera and rHuTNF (data not shown).

**Effect of L-MTP-PE Combined with DOX on the Number of WBCs and Monocytes.** The mean number of circulating WBCs before DOX plus L-MTP-PE treatment was 11,600 ± 5,300/μl. This number was slightly decreased but still within the normal range (5,000–17,000/μl) between days 3 and 7 of treatment (7,400 and 5,600/μl, respectively). Fewer numbers of circulating monocytes were found on day 3 (88 ± 97/μl) post-DOX plus L-MTP-PE treatment, returning to pretreatment counts by day 7 (520 ± 230/μl) (Table 1), which is 3 days earlier than that of dogs treated with DOX alone. Dogs treated with L-MTP-PE alone had no difference in the number of total WBCs or monocytes before and during treatment (data not shown).
DISCUSSION

Many reports have shown that DOX can activate or augment monocytes/macrophages and natural killer cells both in vitro and in vivo to a tumoricidal state (19–29). This study demonstrated that DOX in vivo but not in vitro resulted in the release of a cytotoxic substance (TNF) as measured in the serum and supernatants of the cultured monocytes. Also, our in vitro experiment indicated that DOX (all concentrations) had no effect on the ability of MDP and LPS to activate canine monocytes. The concentrations of DOX tested in vitro are comparable to plasma levels observed in dogs receiving a therapeutic dose of DOX (1 and 24 h postinjection ranged from 300–500 ng/ml and 50–100 ng/ml, respectively) (33, 34). Our data suggest that DOX will not interfere with monocyte activation in vivo by immunomodulators such as MDP ± LPS.

In our in vivo studies, monocyte supernatant-mediated cytotoxicity was enhanced at days 3 and 7 after administration of DOX (Fig. 3). These results are consistent with those previously reported in mice and humans (20, 21, 35). Although the exact mechanism of the tumoricidal effect of monocytes or macrophages exposed to DOX in vivo is still unclear at this point, this phenomenon could be explained in several ways. DOX could act in the same way as many bacteria and bacterial products such as LPS or cytokines to directly activate monocytes/macrophages. Both Hisano and Fidler (35) and Salazar and Cohen (36) were able to show this direct effect on macrophages by DOX (35, 36). A second explanation for the cytotoxic activity of monocytes on tumor cells could be an indirect activation of monocytes by lymphokines released from lymphocytes activated by DOX (22, 37). This hypothesis is based on reports from other investigators that DOX can induce production of lymphokines such as IL-2, interferon, and lymphocyte-activating factor from T lymphocytes (22, 37, 38). Whether this mechanism can explain our in vivo findings of enhanced killing by supernatants of monocytes exposed to DOX is only speculative at this time and requires further study.

The ability of DOX to augment canine monocyte supernatant-mediated cytotoxicity in our studies is consistent with previous findings. In some studies, it has been shown that splenectomy and treatment with anti-macrophage agents such as silica or carrageenan abrogated the antitumor effects of DOX, suggesting that DOX activates macrophage cytotoxic activity (39, 40). In addition, monocyte activation was found in human patients after treatment with DOX (41). In previous studies, DOX augmented cytotoxic T-cell killing of allogeneic tumor cells (24, 26). Similarly, a single injection of DOX enhanced IL-2 production and cell-mediated cytotoxicity of peripheral blood mononuclear cells in human cancer patients (21, 37). In addition, it was found that DOX administration to mice activated natural killer cell activity (22, 25, 28) and cytolytic T-cells (42). Our in vivo studies provide additional evidence that the effect of DOX on the immune system may play a role in tumoricidal activity.

We observed that the single injection of DOX alone led to a slightly transient decrease in the number of circulating monocytes and WBCs at day 3 postadministration; cell number recovered at day 10 and day 14, respectively. When dogs received DOX with L-MTP-PE, the monocytes and WBCs recovered 3 and 4 days faster than they did in dogs receiving DOX alone. These findings agree with the mouse studies previously reported by Hisano and Fidler (35), which demonstrated that the decrease in number of macrophages could be reversed by the i.p. injection of liposomes containing MTP-PE. These findings suggest that MTP-PE may enhance leukocyte recovery in the bone marrow. In our study, monocytes that were harvested at those days showed increased cell-mediated cytotoxicity. Our results indicate that, although DOX slightly depressed the number of recoverable WBCs and monocytes, it did not suppress monocyte tumoricidal potential.

We also observed that peripheral blood monocytes from normal dogs treated with a standard therapeutic dose of DOX could still be activated in vitro by MDP/LPS to lyse tumor targets. This suggests that chemotherapeutic agents such as DOX could be successfully combined with immunotherapeutic agents such as L-MTP-PE in preclinical or clinical trials in dogs.

L-MTP-PE has been shown to enhance killing of a human melanoma cell line (A375) by monocytes and macrophages obtained at 24 and 48 h after DOX administration (7, 10). Although we failed to demonstrate in vivo augmentation of monocyte cytotoxicity at 24 h post-L-MTP-PE, when peripheral blood monocytes were collected at 2 h post-L-MTP-PE treatment, we did observe increased monocyte-mediated cytotoxic activity after L-MTP-PE treatment at days 0, 7, and 14. Our results also showed that in dogs given DOX combined with L-MTP-PE, monocyte-mediated cytotoxic activity at 2 h post-L-MTP-PE injection could be further enhanced compared with those dogs receiving L-MTP-PE alone. Furthermore, augmented cell supernatant-mediated cytotoxicity in monocytes from dogs given the combination of DOX and L-MTP-PE was observed at day 3 through day 10. It would be worthwhile to evaluate the effects of L-MTP-PE on in vivo monocyte function by using other tumor cell lines as targets and to measure other cytokines such as IL-1, IL-6, and interferons that may be released from dogs treated by L-MTP-PE.

Since TNF is an important antitumor cytokine, and we have found in previous studies that L-MTP-PE can enhance serum TNF activity after i.v. injection (11), we again monitored serum TNF activity of dogs at various times after L-MTP-PE alone or L-MTP-PE plus DOX injection. Our results showed that serum TNF activity rapidly increased to peak level at 2 h after the first administration of L-MTP-PE alone, but this response was not observed in the following treatments with L-MTP-PE. However, on subsequent L-MTP-PE injections, monocyte activation at 2 h posttreatment was demonstrated (Fig. 4). When DOX was injected along with L-MTP-PE, levels of serum TNF at 2 h were enhanced and sustained for a longer period of time. These data indicate that serum TNF production after L-MTP-PE injection is most probably, if not all, governed by the release of TNF from monocytes and probably macrophages. These results suggest that DOX may enhance the effect of L-MTP-PE in canine monocyte activation.

The reason that repeated injections of L-MTP-PE alone failed to enhance TNF production in serum is unclear. Studies in human patients receiving L-MTP-PE have shown that serum TNF levels are the highest after the first injection and then decline to nearly pretreatment levels on subsequent injections (43, 44). Biological response modifiers as adjuvant therapy are currently being investigated in many treatment regimen for a variety of tumor models. Activation of monocyte-mediated cytotoxicity by liposome-encapsulated immunomodulators is a particularly attractive therapy for metastatic pulmonary disease in patients with cancer such as osteosarcoma because it is a highly effective way to deliver immunomodulators to macrophages in the pulmonary parenchyma (1). We have shown that in dogs with spontaneous osteosarcoma, treatment with surgery followed by injections of L-MTP-PE significantly delayed or prevented the development of metastases and prolonged survival compared to dogs receiving surgery alone (12–14). Although this therapeutic approach is effective, over half of the dogs eventually died of metastatic disease. Thus, development of new strategies is needed to improve the efficacy of L-MTP-PE treatment. It has been shown that the ratio of monocytes to tumor cells, the state of activity of the monocytes, and the rate of tumor proliferation are critical for successful tumor eradication (1, 7). If the number of tumor cells is far greater than the number of monocytes and macrophages in the tumor location, the effector cells may be inadequate to handle the tumor burden. Therefore, if the tumor burden can be reduced to a level low
enough for the activated monocytes and macrophages to destroy the surviving tumor cells, we could significantly enhance the antimetastatic activity of L-MTP-PE and prolong survival. The combination of existing standard chemotherapeutic agents, such as DOX, which is capable of directly killing tumor cells and perhaps enhancing host immunity, with immunomodulating agents such as L-MTP-PE, may further contribute to prolonged disease-free survival.

In summary, our in vivo results indicate that DOX, administered with L-MTP-PE, enhanced canine monocyte activation induced by L-MTP-PE alone. These data are encouraging, because they suggest that DOX and L-MTP-PE therapy may be combined in the early treatment of cancer patients.

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In Vitro and in Vivo Effect of Doxorubicin Combined with Liposome-encapsulated Muramyl Tripeptide on Canine Monocyte Activation

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