Induction and Maintenance of Monocyte Cytotoxicity during Treatment with Liposomes Containing Muramyl Tripeptide despite Tachyphylaxis to the Cytokine Response

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ABSTRACT

Monocyte-mediated cytotoxicity (determined in a 72-h release assay) and the circulating levels of tumor necrosis factor α (TNF-α), interleukin (IL) 1β, IL-6, IFN-γ, C-reactive protein, and β2-microglobulin were determined in 14 melanoma patients treated with multilamellar vesicle liposomes containing muramyl tripeptide phosphatidylethanolamine, 4 mg twice a week for 12 weeks. Monocyte-mediated cytotoxicity increased 24 h after the first infusion in 9 of 14 patients and had reached maximum levels (mean, 44% ± 8) in all patients by the sixth week; similar values were observed at the 12th week. Once increased in vivo, peripheral blood monocyte cytotoxicity was not susceptible to any further increase after a subsequent in vitro incubation of the monocytes with liposomes. However, the peripheral blood monocytes which were not cytotoxic in vivo were activated by in vitro incubation with liposomes and not by medium. TNF-α and IL-6 peaked 2 h after the first infusion and returned to baseline values at 24 h; they were not significantly increased by subsequent treatments. The induction of fever in patients, observed 2 h after the first infusion, correlated with TNF-α and IL-6 levels. Similarly, C-reactive protein levels also increased at 24 h, but only after the first dose. No increase in β2-microglobulin and IL-1β levels was observed, and IFN-γ was never detected in serum. Two patients experienced stable disease lasting 7 and 12 months, and 12 patients progressed.

These results show that muramyl tripeptide phosphatidylethanolamine administration activates monocyte cytotoxicity and cytokine production (TNF-α, IL-6). Chronic treatment with multilamellar vesicle muramyl tripeptide phosphatidylethanolamine results in tachyphylaxis in terms of cytokine secretion but not cytotoxicity. There was no difference between the maximum cytotoxicity levels obtained in vivo and those obtained in vitro using the same agent. A better understanding of immunoregulation is required for a rational application of this and related immunotherapies.

INTRODUCTION

In experimental models, PBM3 and tissue macrophages activated by liposomes containing muramyl tripeptide become cytotoxic and are capable of destroying tumor cells regardless of their heterogeneity or their resistance to other immune cells or anticancer drugs (1–3). We have recently demonstrated that activated monocytes from cancer patients are capable of recognizing and destroying autologous tumor cells in vitro (4). Because cultured tumor cells may differ from their progenitors as a consequence of cultivation conditions, we have also shown that these monocytes can lyse both cultured and freshly dissociated autologous tumor cells equally effectively. Together, these data support the clinical study of the administration of MLV MTP-PE for the in vivo activation of monocytes in patients with metastatic melanoma.

A number of Phase I studies (5–7) have shown that treatment with liposomes containing muramyl tripeptide is safe and that the optimal immunomodulating dose ranges from 0.5–2 mg/m², far below the maximum tolerated dose (6 mg/m²). In these and subsequent studies (8–11), the substantial in vivo biological activity of MLV MTP-PE has been shown to lead to increased PBM cytotoxicity and the induction of various cytokines. However, the clinical efficacy of this therapy has been disappointing in patients with melanoma, renal cell carcinoma, colorectal tumors, and other tumors, with the possible exception of osteosarcoma in a neoadjuvant setting (10). One reason for the lack of clinical efficacy could be an insufficient activation of PBM in vivo, and, therefore, suboptimal cytotoxicity.

Consequently, the aim of the present study was to evaluate the in vivo levels of PBM-mediated cytotoxicity observed in patients treated with MLV MTP-PE and the possibility of further increasing this cytotoxicity by means of the in vitro re-stimulation of PBM using the same drug. The serum levels of the cytokines usually involved in the monocyte activation and tumoricidal activity were also analyzed in an attempt to discover whether there was a correlation between in vivo and in vitro PBM-mediated cytotoxicity.

MATERIALS AND METHODS

Patients and Study Design. Fourteen patients (5 male and 9 female; median age, 61 years; range, 43–77) with ad-
vanced unresectable malignant melanoma entered the study. Predominant lesions were lung, cutaneous, and lymph nodal.

All pretreatments (chemotherapy plus radiotherapy in two patients, chemotherapy plus immunotherapy in two patients, and immunotherapy alone in two patients) were suspended at least 1 month before the initiation of the study therapy, which consisted of i.v. infusion of MLV MTP-PE, 4 mg twice a week for 12 weeks. The patients were evaluated for clinical response after 12 weeks of treatment, except in the case of early progression requiring the interruption of the treatment.

The biological activity of MLV MTP-PE was evaluated in blood samples drawn before and at 2 and 24 h after the 1st, 12th, and 24th infusion.

The clinical study and related investigations have been approved by the Institutional Review Board and the Ethical Committee of the Centro di Riferimento Oncologico, Aviano. Testimonial oral informed consent was obtained from each patient enrolled in this study.

Serum samples from six healthy donors (3 male and 3 female; median age, 52 years; range, 28–65) were used as normal controls for cytokine studies.

**Preparation of MLV MTP-PE (CGP19835A).** Freeze-dried liposomes containing MTP-PE were supplied by Ciba-Geigy, Ltd. (Basel, Switzerland). Before administration, PBS was added to the vials containing lyophilized lipids and 4 mg MTP-PE. After hydration, the vials were vortexed at high speed for 1 min. Before administration, the preparation was added to 50 ml normal saline.

**Assay for Monocyte Tumoricidal Activity.** Cytotoxicity was assayed by measuring the release of $^{111}$In (12) against human A375 melanoma target cells before and then at 2 and 24 h after the infusions. Peripheral blood mononuclear cells from patients and control healthy donors were separated by means of centrifugation on Ficoll gradients (Lymphoprep; Nyegaard & Co., Oslo, Norway). PBMs were isolated from the mononuclear cells using a Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) discontinuous gradient centrifugation. These cells were then plated ($1 \times 10^5$ monocytes/well) for 1 h in flat-bottomed 96-well plates, after which the nonadherent cells were removed. At this point, the purity of the monocytes was >95% (as determined by nonspecific esterase staining and morphological examination). $^{111}$In-oxine-labeled A375 melanoma tumor cells were then added to produce an initial monocyte to tumor cell ratio of 10:1. After 24 h, the cultures were washed to remove nonadherent and dead cells, refed with fresh medium, and then recultured for an additional 2 days. Seventy-two h after the addition of the tumor cells, the cultures were washed twice with PBS. The adherent viable cells were then lysed with 0.1 ml of 1 M HCl, and their radioactivity was measured in a gamma counter. The percentage of cytotoxicity mediated by activated human monocytes was calculated as follows:

$$100 \times \frac{A - B}{A} = \text{percent specific lysis}$$

where $A$ represents the cpm in the cultures of target cells alone, and $B$ represents the cpm in the cultures containing both test monocytes and target cells.

**In Vitro Monocyte Activation.** Purified monocyte monolayers were incubated at 37°C for 24 h with 0.2 ml control medium or 0.2 ml MLV MTP-PE (50 nmol/ml). After incubation, the adherent monocyte cultures were thoroughly washed with medium, and labeled target cells were added in order to determine monocyte-mediated cytotoxicity.

**Cytokine Assay.** The serum levels of TNF-α, IL-1β, IL-6, and IFN-γ were measured prior to treatment and then at 2 and 24 h after the 1st, 12th, and 24th infusion. The serum was collected and stored at −20°C. TNF-α and IL-6 were measured using an ELISA kit from Biosource International (Camarillo, CA); IL-1β levels were measured by means of an ELISA kit from Amersham (Aylesbury, United Kingdom); and IFN-γ concentrations were determined using an enzyme-amplified sensitivity immune assay kit from Medgenix (Fleurus, Belgium).

The lower limits of assay sensitivity were 4 pg/ml for TNF-α, 2 pg/ml for IL-6, 0.3 pg/ml for IL-1β, and 0.03 IU/ml for IFN-γ.

**CRP and β₂-Microglobulin.** Serum CRP levels were measured using rate nephelometry (015–245559; Beckman Instruments, Inc., Brea, CA). The lower limit of assay was 4 μg/ml.

β₂-microglobulin was assayed by means of a Pharmacia (Uppsala, Sweden) kit, using a double-antibody radioimmunoassay competitive binding method. The lower limit of assay was 0.2 μg/ml.

**Statistical Analysis.** For the comparison of data sets, the analysis was performed by one-way ANOVA for repeated measures and Fisher’s Pooled Least Significant Difference (PLSD) test.

**RESULTS**

**Monocyte Cytotoxicity.**

In Vitro. Monocytic tumor cell line was evaluated prior to therapy and at 24 h after the 1st, 12th, and 24th infusion. At the beginning of the study, PBM cytotoxicity was also assessed 2 h after the infusions, but, at this time point, the number of circulating monocytes was insufficient for determining cytotoxicity because of the marginalization effect of therapy.

An increase or a decrease in monocyte cytotoxicity was defined as a variation of more than 10 in the absolute cytotoxicity levels, according to the literature (8, 11). The detailed results for each patient and cumulative results for all patients are shown in Fig. 1 and Table 1.

Pretreatment PBM cytotoxicity was low (mean, 1% ± 3) in 10 and high (mean, 21% ± 9) in 4 patients. Twenty-four h after the first infusion, cytotoxicity increased to 32% ± 15 in 7 of the 10 patients with low pretreatment values (no change was observed in the remaining 3) and to 49% and 52% in 2 of the 4 patients with high pretreatment values (decreased or unchanged cytotoxicity was observed in the remaining 2).

In all patients, mean PBM cytotoxicity before the first infusion was 7% ± 10, but this had significantly increased to 26% ± 19 ($P = 0.004$) 24 h later. Two patients were withdrawn from the study after 4 weeks for early progression. In the remaining 12 patients, the ANOVA of PBM cytotoxicity after 6 weeks of treatment revealed highly significant differences ($P =$
PBMs, isolated from patients before and after the 1st, 12th, and 24th infusion, were tested for cytotoxic activity either immediately or 24 h after restimulation in vitro with the same drug or with medium. Cumulative results of PBM-mediated cytotoxicity were reported for each patient and as mean percentages of cytotoxicity ± SD.

Table 1  Cumulative results of PBM-mediated cytotoxicity (%) before and 24 h after in vivo infusions of MLV MTP-PE and after restimulation in vitro with the same drug. Data are reported as the mean percentage of cytotoxicity ± SD.

<table>
<thead>
<tr>
<th>Infusion</th>
<th>In vivo</th>
<th>In vitro liposomes</th>
<th>In vitro medium</th>
</tr>
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<tbody>
<tr>
<td>1st (14 patients)</td>
<td>0 h</td>
<td>7 ± 10</td>
<td>30 ± 14</td>
</tr>
<tr>
<td>12th (12 patients)</td>
<td>24 h</td>
<td>26 ± 19</td>
<td>31 ± 10</td>
</tr>
<tr>
<td>24th (8 patients)</td>
<td>0 h</td>
<td>20 ± 25</td>
<td>23 ± 14</td>
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<td></td>
<td>24 h</td>
<td>41 ± 12</td>
<td>48 ± 7</td>
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"PBMs, collected before and after the 1st, 12th, and 24th infusion, were tested for cytotoxic activity either immediately or 24 h after incubation with MLV MTP-PE (50 nmol/ml) or medium. Monocyte-mediated cytotoxicity was determined in a 72-h in vitro release assay as described in Materials and Methods. Data are reported as the mean percentage of cytotoxicity ± SD.

In particular, mean cytotoxicity 24 h after the 12th infusion was 44% ± 8, a significant increase over both the mean value at 0 h (22% ± 13) and the corresponding pretreatment value (6% ± 7).

Similar results were observed at the end of the treatment in eight patients (four were withdrawn from the study because of progressive disease). In these patients, PBM cytotoxicity was significantly increased by treatment (P = 0.0002). In particular, mean cytotoxicity after the 24th infusion was 41% ± 12 and was significantly increased over the mean value at 0 h (20% ± 25) and the corresponding pretreatment value (9% ± 7). The high SD at the beginning of the 24th infusion is due to the fact that 4 of the 8 patients exhibited 0% of specific cytotoxicity.

Due to the large number of comparisons, the probability of having at least one falsely significant result, given the null hypothesis is true, is larger than α. Since no formal adjustment of the α levels of the individual tests is made, all significant comparisons have to be interpreted with care; however, the statistical power of the ANOVA test appears to be strong enough to support these data.

PBM cytotoxicity was also evaluated after the 24-h in vivo incubation with MLV MTP-PE (or medium as control) of the monocytes collected before and after the 1st, 12th, and 24th infusion, in order to determine whether the in vivo activation of PBMs was maximal or still susceptible to a further increase after in vitro restimulation with liposomes.

Our results show that, once increased in vivo, PBM cytotoxicity is not further increased after in vitro restimulation (Table 1). In fact, the in vivo mean pretreatment value was 7% ± 10, which significantly increased to 30% ± 14 (P ≤ 0.05) after in vitro incubation with MLV MTP-PE. This effect was clearly related to the restimulation of liposomes because the in vitro addition of normal medium did not affect the cytotoxicity observed in vivo. On the contrary, 24 h after the first infusion, mean PBM cytotoxicity in vivo was 26% ± 19 and was not susceptible to further increase after in vitro restimulation with...
MLV MTP-PE as well as with medium. Similar results were observed 24 h after the 12th and 24th infusion, when the significant activation of the PBMs was shown by the high level of cytotoxicity and the low SD (44% ± 8 and 41% ± 12, respectively). Therefore, the in vitro restimulation of these monocytes with liposomes did not produce any evident variation in cytotoxicity levels.

Cytokines and Acute Phase Reactants

**TNF-α.** In agreement with the results reported in the literature, circulating TNF-α levels were consistently lower than 10 pg/ml in normal donors. Pretreatment TNF-α serum values were available for 12 patients: they were 0 pg/ml in 7 and 20, 45, 58, 257, and 266 pg/ml in the remaining 5.

Two h after the first infusion, circulating TNF-α levels were only measured in nine patients, eight of whom showed a dramatic increase, which returned to baseline values at 24 h in all patients (Fig. 2). The increase in TNF-α was observed in patients with both low and high pretreatment values of PBM cytotoxicity and, in two patients, it was not associated with any increased PBM tumoricidal activity.

At the 12th and 24th infusion, baseline TNF-α serum levels were low or undetectable, and an increase was observed 2 h after treatment in only 1 patient (Fig. 2).

**IL-6.** The pretreatment values of circulating IL-6 were 0 pg/ml in normal controls and in 10 of 12 patients; the remaining 2 patients presented increased pretreatment serum levels of both IL-6 (325 and 475 pg/ml) and TNF-α (257 and 266 pg/ml).

Two h after the first infusion, IL-6 increased in 4 patients (range, 140–820 pg/ml), decreased in 2 patients, and was undetectable in 3 patients. No data are available for the three remaining patients for this time point. Circulating IL-6 was not detectable 24 h after the first infusion (Fig. 3). High IL-6 serum levels were always associated with high TNF-α levels, and no increase of circulating IL-6 was observed after the 12th and 24th infusion.

**IFN-γ.** No measurable IFN-γ was found in any of the serum samples of nine patients at any time point.

**IL-1β.** Measurable IL-1β (300 and 48 pg/ml, respectively) was detected before the first infusion in 2 of 9 patients, showing also high levels of TNF-α and IL-6. Detectable levels (6–108 pg/ml) of IL-1β were observed before the 12th infusion in 5 of 8 patients and before the 24th infusion in 3 of 4 patients. The increases in IL-1β were randomly observed in different patients, without any apparent association with the other cytokines. No increase in IL-1β values was detected at 2 or 24 h after treatment.

**Acute Phase Reactants.** Serum levels of CRP and β2-microglobulin were measured at 0, 2, and 24 h after the 1st, 12th, and 24th infusion. A marked increase in CRP was consistently noted in all patients 24 h after the first infusion (Table 2). Slight increases in serum CRP were observed in three patients after the 12th infusion and in one patient after the 24th infusion.

No significant changes have occurred in β2-microglobulin levels.

**Toxicity**

The only signs of clinical toxicity were chills and fever ranging from 37.8–39.5°C (mean, 38.5°C), with onset 2 to 4 h after the first infusion. The fever ceased rapidly without any medication in 3–4 h; it reappeared in two patients after the second infusion, but never thereafter.

**Clinical Responses**

Clinical responses to treatment were judged according to WHO criteria. Two patients (one with multiple small lung metastases, the other with multiple s.c. lesions) obtained tumor...
Stabilization lasting respectively 7 and 12 months. All of the remaining patients progressed. Six patients were withdrawn from treatment due to early progression after 8, 9, 12, 14, 15, and 18 infusions.

**DISCUSSION**

The present study evaluates PBM cytotoxicity and cytokine cascade in cancer patients treated with MLV MTP-PE at a dose of 4 mg i.v. twice a week. Previous Phase I studies have shown that the optimal biological dose of MLV MTP-PE in humans ranges from 0.5–2.0 mg/m², which is much lower than the maximum tolerated dose (6 mg/m²; Refs. 5 and 6). Our study shows that the treatment of cancer patients with MLV MTP-PE can induce significant activation of the tumoricidal properties of PBMs. In 9 of our 14 patients, monocyte-mediated cytotoxicity increased over pretreatment values 24 h after the first infusion and had reached maximum levels (mean 44% ± 8) in all patients by the sixth week; similar values were also observed at the 12th week. The progressive increase in PBM cytotoxicity observed during treatment suggests that repeated exposures to MLV MTP-PE may be required to obtain a maximum and prolonged effect on monocytes, which may be either a direct MTP-PE effect or a late effect of secondary mediators.

Once activated, monocytes are reportedly refractory to further activation for 48–72 h (13). In our experience, once increased in vivo, PBM cytotoxicity was not susceptible to any further increase after the subsequent in vitro incubation of monocytes with liposomes. On the contrary, when PBMs were not cytotoxic in vivo, they were activated by in vitro incubation with liposomes but not by medium. Therefore, our data indicate that the treatment at this dose and schedule is able to activate in vivo human monocytes to the same cytotoxicity levels achievable in vitro.

Various studies have shown the powerful synergistic effect of IFN-γ + MLV MTP-PE against tumor cells in vitro (14, 15), including fresh autologous tumor (4). However, in agreement with the findings of other authors (7, 8) circulating IFN-γ was never detected in our patients either before or after the 1st, 12th, or 24th infusion. It would therefore seem that IFN-γ was not responsible for the increased cytotoxicity we observed, since the ability of this cytokine to generate tumoricidal monocytes in vivo is correlated with detectable serum concentrations (16). It is also possible that the cytotoxicity we observed in vivo could have been significantly increased by the presence of IFN-γ, so
combination therapy with IFN-γ plus MLV MTP-PE might improve the tumoricidal activity, and possibly the clinical efficacy, of activated monocytes in cancer patients.

A classic mechanism of cytotoxicity mediated by tumoricidal macrophages is that accomplished by the production of tumor necrosis factor, which has been also shown in some case to be directly cytotoxic on tumor cells (17); this phenomenon, however, is unlikely to be of significance in clinical situations. In eight of the nine patients, we observed a significant increase in circulating TNF-α 2 h after the first infusion and a decrease to baseline values at 24 h. However, a subsequent increase in circulating TNF-α was seen only in 1 of 11 evaluable patients 2 h after the 12th infusion and in none of 8 evaluable patients after the 24th infusion. Similarly, IL-6 serum levels increased only after the first infusion and were always associated with high TNF-α serum levels. It is interesting to note that PBM-mediated cytotoxicity progressively increased with treatment (reaching maximum values after 6 weeks), whereas TNF-α and IL-6 were detected in a significant amount only after the first infusion. Therefore, TNF-α and IL-6 do not appear to be the principal mediators of the increased PBM cytotoxicity observed in vivo.

Why TNF-α and IL-6 did not increase after the 12th and the 24th infusion is not yet understood. Clearly, the pharmacokinetics of TNF-α is very particular: in the sera of patients treated with human recombinant TNF-α in continuous infusion for 5 days, this cytokine becomes undetectable in serum after 6–12 h, despite the duration of the infusion (18). The mechanism of this phenomenon is unknown, although increased TNF-α clearance or a change in vascular leakage are possibilities under consideration. Alternatively, a tachyphylaxis phenomenon could be involved, given that TNF-α inhibitory activity, possibly mediated by soluble fragments of the TNF-α receptors shed by tumor cells (19–22), has been consistently observed in cancer patients. Moreover, just 2 h after the in vitro incubation of monocytes with MLV MTP-PE, there is an early secretion of TNF-α, followed by an increased expression of TNF-α mRNA, which can be detected from 4–24 h (23). It appears, therefore, that the early secretion of TNF-α could derive from a preformed pool of TNF-α present in monocytes, whereas subsequent secretion of TNF-α could be prevented or masked by the tachyphylaxis observed in vivo. It is interesting to note that, in a few patients retreated with MLV MTP-PE after a 2–12-month rest period, plasma TNF-α and IL-6 levels once again increased after the first administration and not thereafter (8).

In response to activation stimuli, monocyte tumoricidal activity and cytokine secretion are not necessarily correlated and, in any case, depend on the nature of the stimuli. For instance, human monocytes activated in vitro with free lipopolysaccharide or muramyl dipeptide produce and release IL-1, but monocytes activated with liposomes containing MTP-PE do not (24). Moreover, Maeda et al. (23) have shown that, following the in vitro interaction of monocytes with MLV MTP-PE, there is an increase in IL-1α and IL-1β mRNA, with the intracellular production of the corresponding cytokines, which is not followed by any extracellular secretion. These data are in agreement with in vivo observations: like most (8, 9), but not all authors (5), we did not observe any increase in IL-1β levels at 2 or 24 h after the 1st, 12th, and 24th infusion. Therefore, the chills and fever observed 2 to 4 h after the first infusion, but only rarely after the second infusion, are reasonably correlated with the serum levels of TNF-α and/or IL-6.

Acute phase reactants are induced by TNF-α and particularly by IL-6 and produced by the liver. An increase in CRP and β2-microglobulin is commonly observed during acute inflammation and monocyte activation. In our patients, only CRP increased 24 h after the first infusion, but not significantly after subsequent infusions: this clearly correlates with the observed levels of circulating TNF-α and IL-6. However, in other studies using MLV MTP-PE with a similar schedule, an increase in CRP (8) or β2-microglobulin (9) was observed throughout the course of treatment, whereas TNF-α and IL-6 increased only after the first infusion. The reason for this discrepancy is not clear, particularly if the reported role of IL-6 as the principal signal for the secretion of acute phase reactants is considered (25).

The treatment of cancer patients with MLV MTP-PE at the dose and schedule used in the present study led to the induction of a number of cytokines and acute phase reactants, and to the activation of PBM to the tumoricidal status. There was no difference between the cytotoxicity levels obtained in vivo and those obtained in vitro using the same agent. Consequently, we can assume that monocytes were maximally activated in patients treated with MLV MTP-PE. However, the fact that the increase in TNF-α and IL-6 was only observed after the first dose (whereas PBM cytotoxicity increased throughout the treatment) indicates that the tumoricidal activity of monocytes can be obtained even in the absence of circulating TNF-α. In the present study MLV MTP-PE effectively induced a sustained activation of monocyte cytotoxicity without tachyphylaxis. In spite of this, no objective responses were noted in this and similar studies. This could depend on the advanced stage of disease of our patients. In fact, a possible clinical efficacy in microscopic disease has been suggested, at least in osteosarcoma (10). Alternatively, or additionally, the synergistic effect of IFN-γ and MLV MTP-PE observed in vitro and the absence of detectable IFN-γ in the sera of our patients suggest that a combination therapy using IFN-γ plus MLV MTP-PE could further improve the tumoricidal activity of PBMs and, possibly, their clinical efficacy.

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REFERENCES

4. Galligioni, E., Quaia, M., Spada, A., Favaro, D., Santarosa, M., Talamini, R., and Monfardini, S. Activation of cytolytic activity in...
osteosarcoma: the cytokine cascade and monocyte activation following
and Vilcek, J. Modulation of monocyte functions by Muramyl tripeptide
6. Creaven, P. J., Cowens, J. W., Brenner, D. E., Dadey, B. M., Han, T.,
5. Murray, J. L., Kleinerman, E. S., Cunningham, J. E., Tatom, J. R.,
Andrejcio, K., Lepe-Zuniga, J., Lamki, L. M., Rosenblum, M. G., Frost,
H., Guterman, J. U., Fidler, I. J., and Krakoff, I. H. Phase I trial of
liposomal Muramyl tripeptide Phosphatidylethanolamine in cancer pa-
6. Creaven, P. J., Cowens, J. W., Brenner, D. E., Dadey, B. M., Han, T.,
Huben, R., Karakousis, C., Frost, H., LeSher, D., Hanagan, J., Andrejcio,
K., and Cushman, M. K. Initial clinical trial of the macrophage activator
Muramyl tripeptide-Phosphatidylethanolamine encapsulated in liposomes
1990.
II, Kedar, I., Creekmore, S., Snol, M., Conlon, K., Kopp, W. C., Huber,
C., Herold, M., Alvord, W. G., Snow, S., and Clark, J. W. Phase I and
immunomodulatory study of a muramyl peptide, muramyl tripetide
8. Kleinerman, E. S., Jia, S. F., Griffin, J., Seibel, N. L., Benjamin,
R. S., and Jaffe, N. Phase II study of liposomal Muramyl tripeptide in
osteosarcoma: the cytokine cascade and monocyte activation following
9. Liebes, L., Walsh, C. M., Chachoua, A., Oratz, R., Richards, D.,
Hochster, H., Peale, D., Marino, D., Alba, S., LeSher, D., Blum, R. H.,
and Vlcek, J. Modulation of monocyte functions by Muramyl tripeptide
Phosphatidylethanolamine in a Phase II study in patients with metastatic
10. Asano, T., and Kleinerman, E. S. Liposome-encapsulated MTP-PE:
a novel biologic agent for cancer therapy. J. Immunother., 14: 286-292,
1993.
11. Fujimaki, W., Itoh, K., An, T., Gano, J. B., Ross, M. L., Mansfield,
P. F. Balch, C. M., Augustus, L. B., Karvevitch, D. D., Johnston, D.,
Fidler, I. J., and Kleinerman, E. S. Cytokine production and immune cell
activation in melanoma patients treated with liposomal Muramyl tripe-
12. Wiltrout, R. H., Taramelli, D., and Holden, H. T. Measurement of
phagocyte mediated cytotoxicity against adherent and non-adherent
target cells by release of 111In oxide. J. Immunol. Methods, 43: 319-331,
1981.
of responsiveness to lymphokines in inflammatory macrophages. Can-
14. Saiki, I., and Fidler, I. J. Synergistic activation by recombinant
mouse interferon-γ and muramyl dipeptide of tumoricidal properties in
15. Sone, S., Tandon, P., Utsugi, T., Ogawara, M., Shimizu, E., Nii, A.,
and Ogura, T. Synergism of recombinant human interferon gamma with
liposome-encapsulated muramyl tripeptide in activation of the tumori-
16. Kleinerman, E. S., Kurzrock, R., Wyatt, D., Quesada, J. R., Gut-
erman, J. U., Fidler, I. J. Activation of suppression of the tumoricidal
properties of monocytes from cancer patients following treatment with
17. Mannel, D. N., Falk, W., and Meltzer, M. S. Inhibition of nonspec-
cific tumoricidal activity by activated macrophages with antiserum
18. Alexander, R. B., and Rosenberg, S. A. Tumor necrosis factors:
clinical applications. In: V. T. De Vita Jr., S. Hellman, and S. A.
Rosenberg (eds.), Biologic Therapy of Cancer, pp. 378-392. Philadel-
and Marten, Y. Characterization of a tumor necrosis factor-alpha
20. Beldegrun, A., Pierce, W., Sayah, D., deKernion, J., Wallach, D.,
Aderka, D., and Figlin, R. A. Soluble tumor necrosis factor receptor
expression in patients with metastatic renal cell carcinoma treated with
Interleukin-2-based immunotherapy. J. Immunother., 13: 175-180,
1993.
Wallach, D., and Kushtai, G. Increased serum levels of soluble receptors
for tumor necrosis factor in cancer patients. Cancer Res., 51: 5602-
22. Landmann, R., Keilholz, U., Scheibenbogen, C., Brockhaus, M.,
Gallati, H., Denz, H., Bargetzi, M., and Ludwig, C. Relationship be-
tween soluble tumor necrosis factor (TNF) receptors and TNFα during
immunotherapy with interleukin-2 and/or interferon-α. Cancer Immu-
23. Maeda, M., Asano, T., and Kleinerman, E. S. Anti-(tumor necrosis
factor) alters the response of human monocytes to liposomal muramyl
24. Tandon, P., Utsugi, T., and Sone, S. Lack of production of Inter-
leukin 1 by human blood monocytes activated to the antitumor state by
liposome-encapsulated Muramyl-tripeptide. Cancer Res., 46: 5039-
5044, 1986.
25. Gauldie, J., Richards, C., Harnish, D., Lansdorp, P., and Baumann,
H. Interferon β2/B-cell stimulatory factor type 2 shares identity with
monocyte-derived hepatocyte-stimulating factor and regulates the major
acute phase protein response in liver cells. Proc. Natl. Acad. Sci. USA,
Induction and maintenance of monocyte cytotoxicity during treatment with liposomes containing muramyl tripeptide despite tachyphylaxis to the cytokine response.

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