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Myelopoietic response in tumour-bearing mice by an aggregated polymer isolated from *Aspergillus oryzae*

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Abstract

The effects of magnesium ammonium phospholinoleate-palmitoleate anhydride (MAPA), a proteic aggregated polymer isolated from *Aspergillus oryzae*, on the growth and differentiation of granulocyte-macrophage progenitor cells (colony-forming unit-granulocyte-macrophage [CFU-GM]) in normal and Ehrlich ascites tumour-bearing mice were studied. Myelosuppression concomitant with increased numbers of spleen CFU-GM was observed in tumour-bearing mice. Treatment of these animals with MAPA (0.5 − 10 mg/kg) stimulated marrow myelopoiesis in a dose-dependent manner and reduced spleen colony formation. No changes were observed in total and differential marrow cell counts. The dose of 5.0 mg/kg MAPA, given prior or after tumour inoculation, was the optimal biologically active dose in tumour-bearing mice and this dose schedule also stimulated myelopoiesis in normal mice. MAPA significantly enhanced survival and concurrently reduced tumour growth in the peritoneal cavity. We propose that the modulatory effect of MAPA on the myelopoietic response may be related to its antitumour activity as a possible mechanism for regulation of granulocyte-macrophage production and expression of functional activities.

Keywords: Ehrlich ascites tumour; Antitumour; Myelopoiesis; MAPA (magnesium ammonium phospholinoleate-palmitoleate anhydride); CFU-GM (colony-forming unit-granulocyte-macrophage)

1. Introduction

The purified compound magnesium ammonium phospholinolate-palmitoleate anhydride (MAPA) is a proteic aggregated polymer (MW = 316 kDa) isolated from *Aspergillus oryzae*. The compound has been shown to possess significant antitumour and antiviral activities in vivo (Durán and Nunes, 1990; Durán et al., 1990, 1993, 1997). Marked inhibition of tumour growth and concomitant lengthening of the host's life span have been observed following MAPA treatment of animals bearing transplantable lymphosarcoma-180, Ehrlich solid carcinoma, plasmacytoma (SP-2/0/Ag14), Walker 256 tumour and spontaneous mammary carcinoma (SP-1) (Durán et al., 1993, 1997).

This compound has also been shown to be non cytotoxic or genotoxic in cultured V79 Chinese hamster fibroblast cells or human lymphocytes, and also non-toxic in mice, dogs and monkeys (Durán et al., 1990, 1993). This is important since the majority of cancer chemotherapeutic agents in routine use are hematotoxic to the recipients, thereby limiting their use in optimal dose schedules. Because no cell growth-inhibiting activity of MAPA in vitro has been shown against 53 tumour cell lines (Durán et al., 1993), it has been suggested that additional factors, particularly immunological support, are involved. Hence, it is of interest to evaluate the actions of this compound on the immune system.

Tumour growth has been reported to induce a variety of phenotypic and functional changes in the cellular constituents of the host's immune system. These changes have been implicated as mechanisms by which tumours subvert potential beneficial host responses. Among the many mechanisms that favour tumour growth, it is postulated that soluble factors generated during tumour growth can

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affect the amount of granulocyte-macrophage progenitors and inhibit various macrophage responses (Roy et al., 1981; Hardy and Balducci, 1985; Kobari et al., 1990; Moore et al., 1992; Bonta and Ben-Efraim, 1993; Hamilton, 1993; Parker and Pragnell, 1995; Elgert et al., 1998).

The Ehrlich ascites tumour, a rapidly growing experimental model, has been found to induce profound alterations in the cell populations of critical importance to ensure host protection, and macrophages are in part responsible for the impaired immune response in tumour-bearing mice (Keeb and Lala, 1978; Pessina et al., 1982a; Parhar and Lala, 1988; Subiza et al., 1989; Fecchio et al., 1990; Kumagai et al., 1995; Segura et al., 1997). Both granulocytes and macrophages are derived from hematopoietic stem cells in the bone marrow. A quantitative measure of the hematopoietic cell proliferative capacities is the colony growth of progenitor cells in vitro in the presence of colony-stimulating factors (CSFs) (Metcalf, 1984). In vitro colony growth may be an early indicator of the hematopoietic changes associated with tumour growth, and in addition, the potential for exhaustion of the hematopoietic system during the host's life span has special significance for cancer patients (Roy et al., 1981; Hardy and Balducci, 1985; Moore, 1992; Parker and Pragnell, 1995).

As bone marrow cell responsiveness to hematopoietic growth factors is an integral part of immune responsiveness, in this work we focused on the effects of MAPA on the growth and differentiation of granulocyte-macrophage colony-forming cells (colony-forming unit-granulocyte-macrophage [CFU-GM]) in normal and Ehrlich ascites tumour-bearing mice. The protective effects of MAPA given prior or after tumour inoculation were investigated. Bone marrow cellularity and cell types present were also assessed. The murine spleen is an important hematopoietic organ along with the marrow. Thus, in order to further investigate the effects of MAPA on extramedullary hematopoiesis, parallel experiments with spleen cells were undertaken in the present study. The survival of these animals was also studied in association with the antitumour potential of MAPA.

2. Materials and methods

2.1. Mice

The mice used in this study were bred at Unicamp Central Animal Facilities (Universidade Estadual de Campinas, Campinas, SP) and raised under specific pathogen-free conditions. Male BALB/c mice, 6–8 weeks old, were matched for body weight before use. Animal experiments were done in accordance with institutional protocols and the guidelines of the Institutional Animal Care and Use Committee.

2.2. Mouse tumour model

Ehrlich ascites tumour was maintained in BALB/c mice by serial transplantation. Tumour cell suspensions were prepared in balanced salt solution at pH 7.4 to final concentrations of 6×10^7 viable cells/ml. In all experimental protocols described, mice were inoculated intraperitoneally (i.p.) on day 0 with 6×10^6 viable tumour cells per mouse in a volume of 0.1 ml. Viability, assessed by the Trypan blue dye exclusion method, was always found to be 95% or more.

2.3. Drug and treatment regimen

MAPA was obtained from selected cultures of *Aspergillus oryzae* and purified by column chromatography according to Durán and Nunes (1990) and Durán et al. (1997). MAPA is a white solid obtained as fine microcrystals in the form of an aggregated polymer after 120 h of culturing in appropriated conditions as previously described (Durán and Nunes, 1990; Durán et al., 1997). The molecular and physicochemical characteristics of MAPA purified from different batches were always reproducible. Each batch of MAPA was evaluated in triplicate to quantify the components of the molecule by quantitative chemical analysis. These analyses provided the following composition: Mg^{2+} $20.1 \pm 0.9\%$, phosphate $45.2 \pm 2.7\%$, palmitoleic acid $2.6 \pm 0.6\%$, linoleic acid $8.7 \pm 0.3\%$, ammonium $10.0 \pm 3.3\%$ and protein $0.49 \pm 0.07\%$. The amino acid composition was also determined by automatic amino acid analysis (Durán and Nunes, 1990; Durán et al., 1997). The chemical structure of MAPA was verified by scanning electron microscopy, X-ray, and infrared and nuclear magnetic resonance spectra. Considering all these analyses, MAPA structure was determined as a magnesium ammonium phosphate with linoleic and palmitoleic acids present as organic anhydrides of phosphoric acid (MW unit: 821) and associated with a basic protein of 10 kDa (Durán and Nunes, 1990; Durán et al., 1997).

The compound was supplied in balanced salt solution at pH 7.4 and diluted immediately before use in appropriate concentrations. Doses of 0.5, 2.0, 5.0, 7.5 or 10 mg/kg were administered for 7 days (consecutive) to groups of normal and tumour-bearing mice by subcutaneously (s.c.) injection of 0.1 ml per mouse. MAPA treatment started 24 h after tumour inoculation and progenitor cell assays were performed on the first day after the last injection. MAPA was also given s.c. prior to tumour inoculation for 7 days (consecutive) at doses of 2.0 or 5.0 mg/kg. The femoral marrow was collected on days 3 and 8 following tumour inoculation for the CFU-GM study. Each experiment included parallel control groups of normal and tumour-bearing mice treated with an equivalent volume of the diluent.

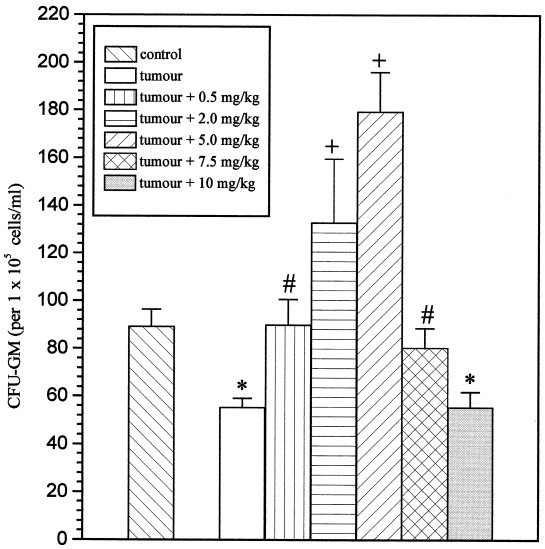

Fig. 1. Bone marrow granulocyte-macrophage colony-forming units (CFU-GM) in mice treated subcutaneously with different doses of MAPA (0.5–10 mg/kg) for 7 days, starting 24 h after the intraperitoneal inoculation of 6×10^6 Ehrlich ascites tumour cells. CFU-GM number was determined on the first day after the last injection of MAPA. Control mice received diluent only. Results represent the means ± S.D. for eight mice. $^*P < 0.01$, compared with control group; $\#P < 0.01$, compared with tumour group; $+\ P < 0.01$, compared with control and tumour groups.

2.4. Hematopoietic stimulator

Recombinant murine granulocyte-macrophage colony stimulating factor (rmGM-CSF) was supplied by Sigma, St. Louis, MO. The rmGM-CSF is an acid glycoprotein of 22 kDa expressed in *Escherichia coli*. Colony formation was stimulated by inclusion in the cultures of 0.025 ng/ml rmGM-CSF when 1×10^5 bone marrow cells were cultured in 1 ml of soft agar. This concentration of rmGM-CSF was determined from the linear portion of the dose-response curve measured in our laboratory before the experiments started.

2.5. Preparation of hematopoietic tissues for progenitor cell assays

After the animals were killed by cervical dislocation, marrow cells were aseptically collected from one complete femur shaft and spleens were removed aseptically. The plug of the marrow was gently extruded into a sterile plastic tube by 1 ml of RPMI 1640 medium (Sigma) injected through the femur. The worm-like marrow plug was then dissociated into a dispersed cell suspension in 5 ml of RPMI medium. As mentioned previously, bone marrow cells were collected at different intervals according to the procedure applied. Spleen cell suspensions were prepared in 5 ml of RPMI medium by gently pressing aseptically removed spleen through a stainless steel mesh net. Spleens were removed on day 8 from mice treated s.c. with 2.0 or 5.0 mg/kg MAPA for 7 days after tumour inoculation.

2.6. Progenitor cell assay

Assays with cell suspensions from femoral marrow and spleen were performed in 1 ml agar cultures in 35-mm petri dishes using 1×10^5 marrow cells or 2.5×10^5 spleen cells per culture. The medium used was Dulbecco's modified Eagle's medium (DMEM, Sigma) containing 20% fetal calf serum and 0.6% agar. Colony formation was stimulated by the addition of rmGM-CSF as described above. The cultures were incubated for 7 days in a fully humidified atmosphere of 10% CO_2 in air and colony formation (clones > 50 cells) was scored at 35 × magnification using a dissection microscope (Metcalf, 1984).

2.7. Bone marrow cellularity

Femoral marrow cells were aseptically collected from mice treated with 5.0 mg/kg MAPA after tumour inoculation. Cytocentrifuge preparations were made of marrow cell suspensions and stained with May–Grunwald–Giemsa. Total and differential cell counts were then performed. Measurements were taken 24 h after the last injection of MAPA.

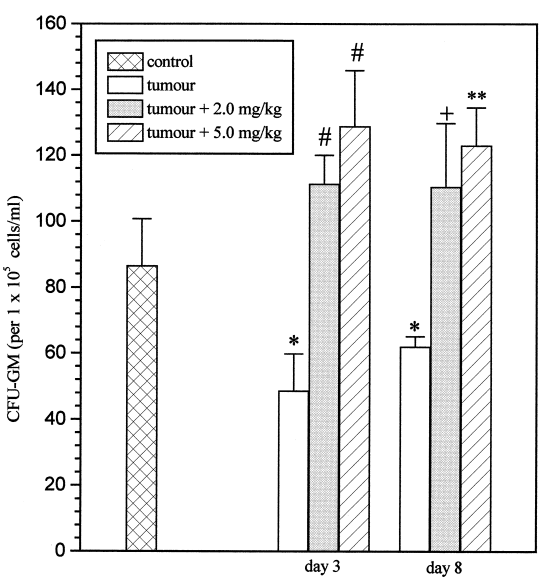

Fig. 2. Bone marrow granulocyte-macrophage colony-forming units (CFU-GM) in mice treated subcutaneously with doses of 2.0 or 5.0 mg/kg MAPA for 7 days previously to the intraperitoneal inoculation of 6×10^6 Ehrlich ascites tumour cells. CFU-GM number was determined on the third and eighth days after tumour inoculation. Control mice received diluent only. Results represent the means ± S.D. for eight mice. $^*P < 0.01$, compared with control group; $\#P < 0.01$, compared with control and tumour-day 3 groups; $+\ P < 0.01$, compared with tumour-day 8 group; $^{**}P < 0.01$, compared with control and tumour-day 8 groups.

Table 1
Effect of 2.0 and 5.0 mg/kg MAPA on spleen granulocyte-macrophage colony-forming units (CFU-GM) in mice bearing the Ehrlich ascites tumour[a]

Group	CFU-GM (per 2.5×10^5 cells)	Inhibition (%)
Control	4.8 ± 3.0	
2.0 mg/kg MAPA	6.1 ± 2.6	
5.0 mg/kg MAPA	7.6 ± 3.7	
Tumour	34.2 ± 6.6[b]	
Tumour — 2.0 mg/kg MAPA	23.6 ± 5.5[c]	31.0
Tumour — 5.0 mg/kg MAPA	22.5 ± 4.7[c]	34.2

[a]Control and Ehrlich ascites tumour-bearing mice were treated with MAPA or diluent for 7 days, starting 24 h after tumour inoculation and, after the last injections, the number of CFU-GM in spleen was determined. Colonies were counted after 7 days of incubation. Results are the means ± S.D. for eight mice.
[b]$P < 0.01$, compared with control group.
[c]$P < 0.01$, compared with tumour group.

2.8. Antitumour evaluation

The antitumour activity of MAPA was evaluated by measuring survival time and tumour growth inhibition. Mice treated with 0.5, 2.0, 5.0, 7.5 or 10 mg/kg MAPA after tumour inoculation were checked daily for survival. The ascitic fluid from the peritoneal cavity of tumour-bearing mice treated with 5.0 mg/kg MAPA was quantitatively isolated by peritoneal lavage 24 h after the administration of the last injection. The total number of tumour cells was counted by the Trypan blue exclusion method.

2.9. Statistical analysis

Comparisons of data among all groups were done by analysis of variance followed by the Tukey test. The Student's *t*-test was used to compare the tumour growth of treated and control tumour-bearing mice. Survival curves were tested by comparing the cumulative percentage of survival using the Gehan–Wilcoxon test. All P values represent two-sided test of statistical significance. Statistical significance was assigned when $P < 0.05$.

3. Results

3.1. Bone marrow and spleen progenitor cell assays

The number of bone marrow granulocyte-macrophage colonies was significantly reduced in tumour-bearing mice ($P < 0.01$) (Figs. 1 and 2). As can be seen in Fig. 1, the administration of 0.5, 2.0 and 5.0 mg/kg MAPA, after tumour inoculation, caused a dose-dependent increase in the number of granulocyte-macrophage colonies in tumour-bearing mice. Exposure of tumour-bearing mice to 2.0 and 5.0 mg/kg led to an increased number of CFU-GM, reaching levels 49% and 99% over control values, respectively ($P < 0.01$). Conversely, 0.5 and 7.5 mg/kg doses brought CFU-GM to values similar to those of controls and the 10 mg/kg treatment did not prevent the myelosuppression induced by the tumour (Fig. 1). In addition, treatment with the same doses of MAPA given for 7 days prior to tumour inoculation resulted in a similar pattern of dose response, with tumour-induced myelosuppression being prevented only at the 2.0 and 5.0 mg/kg doses (Fig. 2). It is interesting to mention that in the groups of normal mice exposed to the same concentrations of MAPA, the increase in colony numbers was only significant following treatment with 5.0 mg/kg ($P < 0.01$) (data not shown), suggesting a stimulatory effect of this biosynthetic compound on normal murine myelopoiesis.

Evaluation of peripheral hematopoiesis, as observed by the numbers of CFU-GM in the spleen, is summarized in Table 1. Tumour inoculation produced a dramatic increase in the number of spleen colonies ($P < 0.01$). This effect was partly, but significantly, reversed by treatment of these animals with 2.0 and 5.0 mg/kg MAPA for 7 days following tumour inoculation ($P < 0.01$), resulting in 31% and 34% inhibition, respectively, with no major differences between the counts for the two doses tested. No statistical differences were observed in the number of CFU-GM in the spleen of normal treated mice compared with the control animals (Table 1). The changes in spleen colony formation in tumour-bearing mice after administration of MAPA suggest a shift towards the normal pattern following treatment.

Table 2
Changes in cell populations in the bone marrow of mice bearing the Ehrlich ascites tumour after treatment with 5.0 mg/kg MAPA[a]

Parameters ($\times10^6$)	Control	Tumour	Tumour — 5.0 mg/kg
Femoral marrow cell counts	10.6 ± 2.6	8.6 ± 2.2	12.3 ± 3.3
Lymphoblasts	2.1 ± 0.2	1.3 ± 1.4	1.7 ± 0.7
Myeloblasts	0.9 ± 0.2	0.5 ± 0.3	0.5 ± 0.2
Promyelocytes and myelocytes	1.7 ± 0.1	1.0 ± 0.4	1.2 ± 0.6
Metamyelocytes and neutrophils	6.4 ± 0.3	4.2 ± 1.3	5.3 ± 1.4

[a]Mice inoculated with Ehrlich ascites tumour cells were treated with MAPA or diluent for 7 days, starting 24 h after tumour inoculation and, after the last injections, measurements were taken. Results are the means ± S.D. for eight mice.

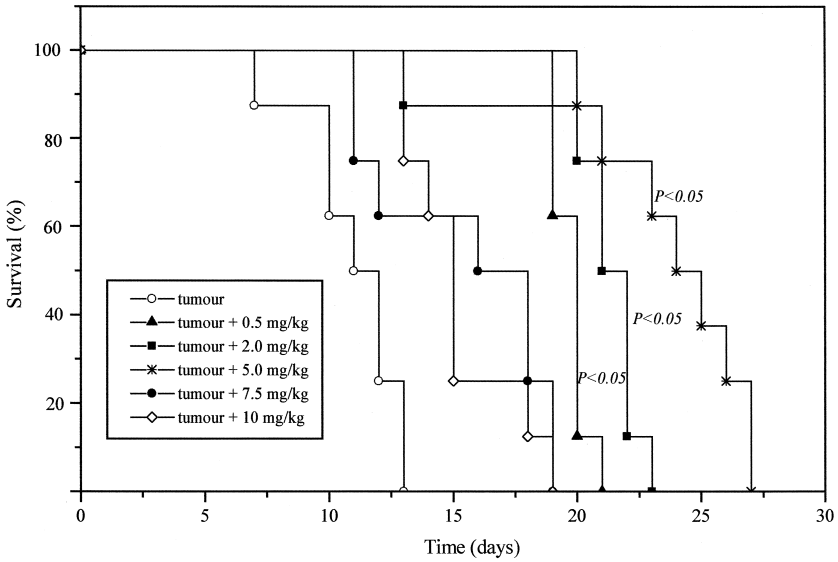

Fig. 3. Effect of different doses of MAPA on the survival of tumour-bearing mice. Mice were treated with subcutaneous injections of 0.5–10 mg/kg MAPA for 7 days, starting 24 h after the intraperitoneal inoculation of 6×10^6 Ehrlich ascites tumour cells. Control mice received diluent only. Groups of eight mice were checked daily for their rate of survival. $P < 0.05$, compared with tumour group.

3.2. Bone marrow cellularity

As can be seen in Table 2, no consistent differences were observed in total femur cell counts or in the composition of the bone marrow population due to MAPA treatment or tumour inoculation. Cell numbers, and particularly metamyelocyte and neutrophil numbers, were in fact marginally lower in the marrow of tumour-bearing mice than in control mice, but in no instance were these differences statistically significant.

3.3. Antitumour evaluation

The effects on survival of the administration 0.5–10 mg/kg MAPA after tumour inoculation are presented in Fig. 3. All the untreated tumour-bearing mice died within 13 days, whereas treatment with 0.5, 2.0 and 5.0 mg/kg MAPA significantly enhanced the rate of survival to 21, 23, and 27 days, respectively ($P < 0.05$). Higher doses (7.5 and 10 mg/kg) did not significantly affect survival.

As can be seen from Table 3, the administration of 5.0 mg/kg of MAPA after tumour inoculation resulted in a significant inhibition of tumour growth ($P < 0.05$), as evident from a 50% reduction in intraperitoneal tumour cell burden on day 8.

Table 3
Tumour growth inhibition after treatment with 5.0 mg/kg MAPA[a]

Group	Ascites cells ($\times 10^8$)	Inhibition (%)
Tumour	12.7 ± 4.2	
Tumour — 5.0 mg/kg	6.3 ± 2.0[b]	50.0

[a] Mice inoculated with Ehrlich ascites tumour cells were treated with MAPA or diluent, for 7 days, starting 24 h after tumour inoculation and, after the last injections, measurements were taken. Results are the means ± S.D. for eight mice.

[b] $P < 0.05$, compared with tumour group.

4. Discussion

Depletion of early hematopoietic progenitors or inhibition of hematopoiesis itself in both human and animal tumour systems may represent an acute effect of rapidly growing tumours. In the present study, we observed that the number of CFU-GM in the bone marrow of mice bearing the Ehrlich ascites tumour was reduced, whereas the number of CFU-GM in the spleen was increased. The administration of MAPA to tumour-bearing mice at doses varying from 0.5 to 7.5 mg/kg led to stimulation of myelopoiesis in a dose-dependent manner. The optimal biologically active dose of MAPA (5.0 mg/kg), given either prior or after tumour inoculation, increased the number of CFU-GM in the bone marrow without increasing the total number of bone marrow cells or the composition of its subpopulations. Similarly, the dose of 5.0 mg/kg MAPA also stimulated myelopoiesis in the bone marrow of normal mice. Conversely, this compound hardly affected the number of CFU-GM in the spleen and slightly reduced the number of CFU-GM in Ehrlich ascites tumour-bearing mice at both doses used (2.0 and 5.0 mg/kg).

A variety of mechanisms could be postulated to explain the alterations produced in the CFU-GM compartment. Cancer cells routinely circumvent macrophage cytotoxicity and redirect macrophage activities to promote tumour development (Fecchio et al., 1990; Watson et al., 1991; Koo et al., 1992; Murai et al., 1995; Segura et al., 1997; Elgert et al. 1998). Our results concerning the effects of tumour

load on myelopoiesis provide additional evidence to the documented ability of the Ehrlich ascites tumour to affect the receptor system of granulocyte-macrophage progenitor cells and a number of the functional capabilities of macrophages (Keeb and Lala, 1978; Pessina et al., 1982a,b; Parhar and Lala, 1988; Subiza et al., 1989; Fecchio et al., 1990; Segura et al., 1997). Apparently, stimulation of myelopoiesis in the spleen of Ehrlich ascites tumour-bearing mice is secondary to the tumour. One of the most important aspects of the influence of tumour carriage in malignant ascites is the aggressive behaviour of tumour-derived factors that stimulate the proliferation of splenic macrophages with suppressive activity (Keeb and Lala, 1978; Pessina et al., 1982a,b; Hardy and Balducci, 1985; Parhar and Lala, 1988; Kobari et al., 1990; Watson et al., 1991; Oghiso et al., 1993; Yamamoto et al., 1995). In this respect, Tomida et al. (1984) have reported that Ehrlich tumour cells produce factors capable of inducing proliferation of spleen cells with phenotypic and functional changes.

Accumulating evidence points to the role of inhibitory factors such as prostaglandin E_2 and cytokines, produced by macrophages and the tumour itself, in the myelosuppression of tumour bearing animals (Pessina et al., 1982a; Bonta and Ben-Efraim, 1993; Hamilton, 1993; Young, 1994). Fecchio et al. (1990) have demonstrated that the intraperitoneal inoculation of Ehrlich ascites tumour cells induces the release of increased levels of prostaglandin E_2 and a delayed inflammatory response. Increased macrophage production of prostaglandin E_2 down-regulates T-cell proliferation and blocks the stimulatory action of tumour necrosis factor-α on macrophages (Alleva et al., 1993b). Additionally, it has been demonstrated that prostaglandin E_2 favours T helper 2-like cytokine secretion profiles in murine and human T-cells by inhibiting the production of the T helper 1-associated cytokines interleukin-2 and interferon-γ (Betz and Fox, 1991; Hilkens et al., 1995). A similar process has been described with cytokines such as transforming growth factor-β, interleukin-10 and interleukin-6, which are produced mainly by tumour cells, causing a down-regulatory effect on the production of interferon-γ and tumour necrosis factor-α by macrophages (Yamamoto et al., 1995; Elgert et al., 1998). Recent work has shown the presence of transforming growth factor-β precursors in Ehrlich ascites tumour cells as well as in ascitic fluid of tumour-bearing mice (Segura et al., 1997), but no receptors for this cytokine were detected in these tumour cells (Elexpuru et al., 1997). Therefore, the limitation of myelopoiesis in the tumour-bearing state may be explained partly by increased production of prostaglandin E_2 and possibly other secretory products, which would be expected to predominate when macrophages are affected by external influences such as tumour load.

Our present data showed that the dose of 5.0 mg/kg MAPA elicited optimal modulatory effects on bone marrow and spleen colony formation. In addition, this dose, given after tumour inoculation, doubled the life span of Ehrlich ascites tumour-bearing mice compared with that of the untreated control mice. Concurrently the number of tumour cells in the peritoneal cavity was reduced by 50%. These results suggest that the antitumour activity of MAPA might be related to some ability of the compound to keep the balance between positive and negative stimuli controlling myelopoiesis, which would be expected to result from changes in the local behaviour of various cell types, particularly T-cells and macrophages. It is likely that doses higher than 5.0 mg/kg of the compound could not counteract the progressive increase in the macrophage–prostaglandin E_2 production induced by the tumour. However, we could not exclude the possibility that higher doses of MAPA may lead to stem-cell depletion similar to that observed with positive growth factors used in routine clinical practice to reduce myelosuppression (Hornung and Longo, 1992; Moore, 1992; Parker and Pragnell, 1995). It is noteworthy that interferon-γ, a T helper 1 type cytokine, is a potent macrophage activation molecule that regulates both class II major histocompatibility complex protein Ia expression and prostaglandin E_2 production (Alleva et al., 1993a). Moreover, interferon-γ in cooperation with tumour necrosis factor-α can induce differentiation of transforming growth factor-β-secreting myelomonocytic progenitor cells into nonsuppressive monocytic cells that secrete tumour necrosis factor-α rather than transforming growth factor-β (Young et al., 1997). These observations assume additional significance in view of the ability of tumour necrosis factor-α to inhibit the growth of Ehrlich ascites tumour cells in vitro and in vivo (Fung et al., 1985, 1995; Obrador et al., 1998). Thus, it appears that interferon-γ could play an important role in the mechanism by which MAPA interferes with the tumour-induced myelopoietic perturbations and tumour growth.

Relative to the host's ability to eliminate tumours, disorders of maturation that compromise the full competence of mature macrophages have been reported (Fecchio et al., 1990; Zicari et al., 1992; Bonta and Ben-Efraim, 1993; Barth and Morahan, 1994; Watson and Lopez, 1995). Therefore, it is very likely that the antitumour effect of MAPA, acting synergistically with other factors, such as specific cytokines, may further result from enhanced macrophage activation against Ehrlich ascites tumour cells. In line with this suggestion, the reduction in the number of CFU-GM in the spleen of treated tumour-bearing mice could be ascribed in part to a decreased production of tumour-derived factors responsible for the disturbed hematopoietic activity in this organ. This further supports the idea that macrophage secretion of inhibitory molecules should also be affected by MAPA. Thus, an increased demand for functional cells during cancer might be compensated for by the stimulation caused by MAPA of bone marrow myelopoiesis. Taken together, it is reasonable to assume that the possible therapeutic effect of MAPA against Ehrlich ascites tumour may be produced by the

number of macrophages available and the extent to which their functional activities are modulated.

The findings presented herein show that treatment with MAPA stimulates bone marrow myelopoiesis in mice while it acts against tumour evolution, thus prolonging host survival. Taking into account the limitations of dose schedule, it is encouraging in this context to consider MAPA for combination chemotherapy to protect the host from hematotoxicity as well as to supplement tumouricidal efficacy.

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