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THE POTENTIAL ROLE OF MONOCYTE CHEMOTACTIC AND ACTIVATING FACTOR (MCAF) IN TUMOR GROWTH

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INTRODUCTION

In the course of the studies of a human monocyte derived neutrophil chemotactic factor (MDNCF/IL 8), a distinct chemotactic factor specific for monocytes (MCF) was also detected. We recently succeeded in purifying MCF from the conditioned media of a myelomonocytic cell line (THP-1) (Matsushima et al., 1989) and cloned the cDNA from a library of an activated human monocytic cell line HL 60 (Furutani et al., 1989). In addition to being chemotactic for monocytes, MCF also activates monocytes to be cytostatic for several types of human tumor cells in vitro. Therefore, we termed this cytokine monocyte chemotactic and activating factor (MCAF).

A variety of murine and human tumor cell lines including our fibrosarcoma cell line 8387 as well as cultured baboon aortic medial smooth muscle cells have been reported to produce MCF-activities (Wang et al., 1986, Benomar et al., 1987, Valente et al., 1988). The in vitro production of this MCF correlated with the macrophage content of in vivo tumors. Furthermore, MCF identical to MCAF has been purified from glioblastoma cell lines and PHA-stimulated PBMC (Yoshimura et al., 1989). In the present paper, we have investigated whether the human fibrosarcoma cell line 8387 produces MCAF. We have also evaluated whether MCAF activates macrophages to produce superoxide anion or lysosomal enzyme release from human monocytes, and established whether MCAF has in vivo monocyte recruitment activity. We have discussed the possibility that these MCAF-induced activities may contribute to tumor resistance.

MATERIALS AND METHODS

Purification of MCAF

Supernatants from fibrosarcoma cell cultures stimulated with 100 ng/ml rTNFα overnight were applied to a heparin Sepharose column. MCAF was eluted with 0.05 M Tris-HCl, 0.5 M NaCl and was chromatographically separated using Sephacryl S-200. The fractions which contained monocyte chemotactic activity were pooled and applied to a carboxymethyl-silica gel column (CM-3SW), which was equilibrated with 0.02 M 3-[N-morpholino] propanesulfonic acid (MOPS), pH 6.5. Finally, samples containing monocyte chemotactic activity were applied to a reverse phase chromatography column (TMS-250 Ultropac column) connected to an HPLC system.

Monocyte Chemotaxis Assay

Monocyte chemotactic activity was measured using a 48-well multiwell Boyden chemotaxis chamber as described (Falk et al., 1980). MCAF was serially diluted in RPMI containing 0.5 % bovine serum albumin (BSA) and added to the lower chamber. Mononuclear cells ($2x10^6$) free of neutrophils were added to the upper chamber, that was separated from the lower chamber by a polyvinylpyrrolidone-free polycarbonate membrane with a pore size of 8μm. After 90 minutes at 37°C the membrane was fixed, dried and stained. The migrated cells consisting of more than 98 % monocytes were counted and the migration was expressed as a chemotactic index (CI), which is the ratio of the number of cells that migrated towards MCAF to the number of cells that migrated towards control medium.

Superoxide Anion Assay

Adherent monocytes were incubated with 10 μl of 50 μg/ml cytochalasin B in the presence or absence of superoxide dismutase. To each sample, 50 μl of the diluted stimuli and 10 μl cytochrome C (120 μM) was added and the cells incubated for 30 minutes at 37°C. The absorbance of each sample was read in a multiwell ELISA reader at 550 nm. The protein content was measured and the nmoles of superoxide released was calculated using the formula: (absorbance 550 nm x 100)/(6.3 x mg cell protein).

N-acetyl β-D-glucosaminidase Assay

Adherent monocytes (> 90 % pure) were preincubated with 100 µl of 5 µg/ml cytochalasin B, followed by the addition of the appropriate stimulus for 1 hour at 37°C. Supernatants and the lysed cells were added to 400 µl of the substrate (7.5 mM p-nitrophenyl-acetyl-β-D-glucosamide in 0.125 M citrate buffer containing 0.125 % Triton X-100) for 2.5 hours at 37°C. The amount of enzyme present was defined as that amount of β-N-acetylglucosaminidase that hydrolyzes 1 µmole of substrate/minute. The percentage of enzyme released was calculated using the formula: enzyme content of supernatant x 100/(supernatant content + cell monolayer content).

RESULTS AND DISCUSSION

The production of MCAF mRNA upon stimulation of the human fibrosarcoma cell line 8387 with IL 1 and TNF was examined using Northern blotting analysis. Although low expression of MCAF mRNA was found in unstimulated cells, both IL 1 and TNF, but not LPS, increased the level of expression considerably. TNF consistently induced the production of more monocyte chemotactic activity than IL 1. MCAF was, therefore, purified to homogeneity from 8 liters of TNF (100 ng/ml) stimulated fibrosarcoma cells by sequential chromatography including heparin-Sepharose affinity chromatography, CM-HPLC, and RP-HPLC. Material represented by two small absorbance peaks on the CM-HPLC (fraction 25 and 26) that coeluted with monocyte chemotactic activity and β-glucosaminidase releasing activity was applied to RP-HPLC for further purification. In both RP-HPLC eluates a discrete absorbance peak (CM 25, RP 21-22 and CM 26, RP 22-23) coeluted and contained monocyte chemotactic activity, β-glucosaminidase releasing activity and monocyte cytostatic inducing activity suggesting that all these activities are due to a single molecular species. SDS-PAGE analysis of the purified MCAF showed microheterogeneity in relative molecular weight of about 15 kDa. A synthesized fraction of MCAF was used to generate a rabbit polyclonal antibody to MCAF. This antibody reacted in Western blotting analysis with all bands detected on SDS-PAGE. In addition to dose-dependent monocyte chemotactic and N-acetyl β-glucosaminidase releasing activities which are shown in Table 1, purified MCAF also induced superoxide anion release at concentrations above 20 ng/ml.

TABLE 1. Monocyte Chemotactic Activity and N-Acetyl β-Glucosaminidase Releasing Activity of Purified MCAF (CM 25, RP 21-22).

Concentration (pg/ml)	Monocyte Chemotactic Activity (CI)	N-Acetyl β-Glucosaminidase Release (%)
54000	3.91 +/- 0.59	10.0 +/- 0.6
18000	3.47 +/- 0.39	9.1 +/- 0.5
6000	2.87 +/- 0.53	7.3 +/- 0.5
2000	2.68 +/- 0.47	0.4 +/- 0.2
660	1.81 +/- 0.36	-
220	2.00 +/- 0.43	-
73	1.58 +/- 0.24	-
25	1.26 +/- 0.28	-
fMLP (10^{-7}M)	2.54 +/- 0.37	NT
PMA (20 nM)	NT	30.3 +/- 0.1

The identity of the 8387-derived MCAF to MCAF previously purified from the THP-1 cell line was substantiated by specific inhibition of THP-1 derived ^{125}I-labeled MCAF binding to human PBMC at 37°C by the MCAF enriched unlabeled fractions (CM 25, RP 21-22; CM 26, RP 22-23) as shown in Fig. 1. Both fractions were able to compete with the THP-1 derived MCAF in a dose dependent manner, while another basic polypeptide chemoattractant, IL-8, failed to inhibit the binding of ^{125}I-labeled MCAF to human PBMC at concentrations from 0.1 ng/ml to 10 μg/ml. To evaluate the in vivo effects of 8387-derived MCAF, serial dilutions of MCAF in PBS were injected into the ears of Lewis rats at various time points. MCAF showed exclusive monocyte recruitment activity over a dose range from 0.053 ng/ml to 53 ng/ml when assayed at 3, 6 and 18 hours but not at 3 minutes, 30 minutes, or 90 minutes.

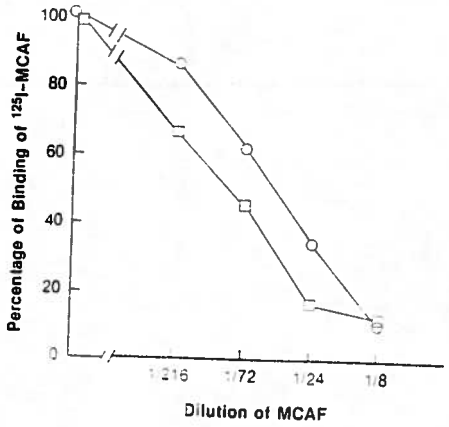

Figure 1. Competition for the binding of ^{125}I-MCAF to human PBMC by unlabeled purified MCAF. -□- CM25, RP 21-22 (10,6 μg/ml) and -o- CM 26, RP 22-23 (6.8 μg/ml). The binding is expressed as a percentage of the binding obtained with THP-1 derived ^{125}I-MCAF alone.

A large number of tumors are infiltrated with macrophages, which may result in either tumor growth by releasing "growth factors" or exert a tumor suppressive effect through the production of "macrophage activating factors" such as MCAF, IFN-α, TNF, or IL 1. Tumor derived cell lines have been shown to produce MCAF and related molecules (Wang et al., 1986, Benomar et al., 1987, Kuratsu et al., 1989). Such tumors are often infiltrated with macrophages in vivo, and are associated with a better prognosis. IL 1 and TNF can induce MCAF in certain tumor cell types, and in normal fibroblasts and endothelial cells. Thus the production of MCAF by the tumor as well as in neighboring tissues may be regulated by TNF and IL 1. MCAF may contribute to control tumor growth by recruiting activated macrophages to the tumor site. This hypothesis is supported in this paper by the ability of MCAF to induce in vivo monocyte recruitment. Furthermore, MCAF also activates macrophage cytostatic activity and induces enzyme release and O_2^- release. The potential utility of these cytokines in cancer treatment remains to be established.

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