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Human Granulocyte-Macrophage Colony-stimulating Factor Is a Growth Factor Active on Human Ovarian Cancer Cells

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Human granulocyte-macrophage colony-stimulating factor (GM-CSF) is a regulatory glycoprotein that stimulates the production of granulocytes and macrophages from committed hematopoietic progenitor cells both *in vitro* and *in vivo*. In this report, we show that recombinant human GM-CSF enhances colony formation by nonhematopoietic human ovarian cancer cell lines, IGROV-1, A2774, ME-180, Pa-1 and A2780. GM-CSF also enhanced the colony formation by cells obtained from fresh ascites of a patient with ovarian mucinous cystadenocarcinoma and a patient with serous papillary ovarian carcinoma. Our observations were made with GM-CSF concentrations between 0.1 to 1 ng/ml; these concentrations are equivalent to the dosages generally used for bone marrow recovery after chemotherapy.

Key words: GM-CSF — Clonal growth — Ovarian cancer cells

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a glycoprotein hematopoietic hormone that stimulates progenitor hemopoietic cells to proliferate and differentiate *in vitro* into mature granulocytes and macrophages.[1)] The biologic actions of GM-CSF are mediated by binding to specific high-affinity receptors found on the surface of myeloid precursors, mature granulocytes and mononuclear phagocytes.[2,3)]

Recently the presence of functional GM-CSF receptors on the surface of nonhematopoietic tumor cells has been demonstrated.[4)] Furthermore, there is experimental evidence that GM-CSF can stimulate the clonal growth of some malignant nonhematopoietic cells *in vitro* (small lung carcinoma[4,5)]; human osteosarcoma[6)]; MCF-7 human breast carcinoma[6)]; human colon adenocarcinoma cell lines[7)]). The growth-stimulating effect of this factor occurred with concentrations of GM-CSF that result in colony formation by normal hematopoietic progenitor cells.[1)] Ovarian cancer cell lines secrete significant amounts of an M-CSF-like factor, which may be involved in tumor cell proliferation, by an autocrine or paracrine mechanism.[8)] Moreover, the ascites fluid obtained from ovarian cancer patients contains putative growth factors with mitogenic properties.[9)] GM-CSF is currently the subject of clinical trials to accelerate the recovery of bone marrow function after cytotoxic, myelosuppressive chemotherapy in patients with malignant tumors.[10,11)]

This report describes the effect of recombinant human (Rh)GM-CSF on the clonal growth of human ovarian cancer cell lines (IGROV-1, A2774, Me-180, Pa-1 and A2780) and on cells obtained from fresh ascites from a patient with ovarian mucinous cystadenocarcinoma (OMC) and a patient with serous papillary ovarian carcinoma (OSC). All cell lines were grown in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum (HI-FCS) (A2780 in 10% FCS). Human ovarian cancer cell lines were: IGROV-1, A2774 (kindly provided by Dr. S. Ferrini, our Institute), A2780 (kindly provided by Dr. A. Mazzoni, Department of Pharmacology, University of Milano, Italy), Pa-1 and Me-180 (ATCC, Rockville, MD); OMC and OSC cells were freshly isolated from ascitic fluid of two patients suffering from ovarian cancer. RhGM-CSF 1.4×10^6 U/ml was obtained from Schering-Plough, Kenilworth, NJ. The human tumor clonogenic assay (HTCA) was previously described.[12)] The cells were plated at concentrations of 100,000 (cell lines) or 300,000 (freshly isolated cells) viable tumor cells per plate in the upper layer of the two-layer agar culture system. The effect of RhGM-CSF, in a range of concentrations from 0.01 to 32.0 ng/ml, was tested by addition to the upper layer and to the agar matrix, to allow continuous exposure during the culture period (average of ten days); aggregates of 20 or more cells were scored as colonies and counted.

RhGM-CSF stimulated clonal growth of all cell lines with dose-response correlations (Fig. 1 and Table I). This was reproducible in three different experiments, performed in six different plates. Significant stimulation of clonal growth was observed at concentrations up to 0.1 ng/ml for IGROV-1, A2774 and PA-1 and up to 1.0

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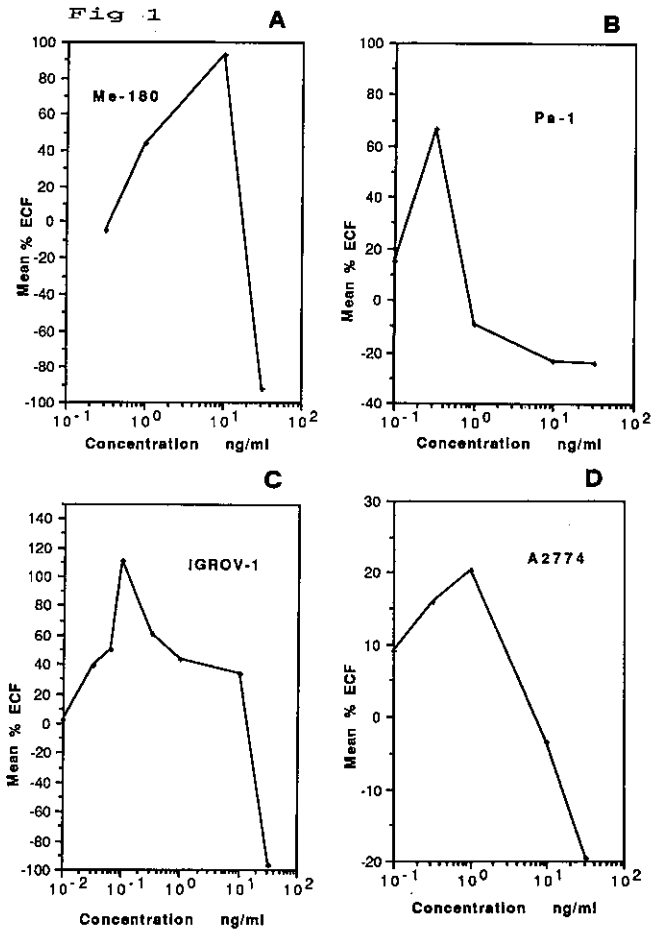

ng/ml for ME-180. This stimulation was maximum at 0.1 ng/ml for IGROV-1, at 1.0 ng/ml for A2774, and at 0.32 ng/ml for PA-1 and then decreased with further increase of RhGM-CSF concentration until a cytotoxic effect was noted at a concentration of 32.0 ng/ml for IGROV-1 and ME-180, and at 10.0 ng/ml for A2774. Only on A2780 cell line did RhGM-CSF fail to show clonal growth potentiation at low concentration, though at 32 ng/ml a potentiating effect was observed.

RhGM-CSF also stimulated the clonal growth of OMC and OSC cells, the greatest stimulation being observed at 10.0 ng/ml for OMC with a bell-shaped dose-response curve as reported for the cell lines tested (Fig. 2). OSC showed maximum clonal growth at 32.0 ng/ml. The high specificity of the stimulation of the clonal growth of IGROV-1 by RhGM-CSF was demonstrated by incubation of the cells with the factor previously boiled for 10 min; under these conditions cultures were not stimulated. These results indicate that a hematopoietic growth factor, GM-CSF, may stimulate the *in vitro* clonal growth of malignant nonhematopoietic cells, such as human ovarian carcinoma cells. This effect is apparent

Fig. 1. Dose-response curves for GM-CSF enhancement of colony formation by 100,000 Me-180 (A), Pa-1 (B), IGROV-1 (C) and A2774 (D) human ovarian cancer cell lines. Colony growth (>20 cells) for all cell lines were enumerated on day 10. Results, expressed as mean % ECF (enhancement of colony formation), are the average of at least three wells (in this and in the following figure).

Table I. Effects of GM-CSF on Tumor Colony Formation of Human Cancer Cell Lines and Tumor Cells from Patients with Ascites and Ovarian Carcinoma

Tumor	GM-CSF (ng/ml) — Mean No. colonies ±SE	0.1 Mean No. col. ±SE	0.1 Mean % ECF[a]	0.32 Mean No. col. ±SE	0.32 Mean % ECF	1.0 Mean No. col. ±SE	1.0 Mean % ECF	10.0 Mean No. col. ±SE	10.0 Mean % ECF	32.0 Mean No. col. ±SE	32.0 Mean % ECF
IGROV-1	134.8±10.3	285.0±11.4	111.4	217.3±9.1	61.0	194.2±11.0	44.0	181.3±15.6	34.0	4.5±1.2	−97.0
A2774	112.7±11.9	123.0±9.3	9.1	130.7±5.0	16.0	135.6±10.1	20.3	110.0±12.8	−3.4	91.7±8.6	−19.6
Me-180	87.3±11.1	—	—	83.3±10.3	−4.6	125.0±13.7	43.2	168.2±16.1	92.7	8.0±1.9	−91.8
Pa-1	49.9±3.2	57.3±3.5	14.8	83.3±4.2	66.9	45.4±5.7	−9.0	38.3±5.6	−23.2	38.0±1.8	−23.8
A2780	24.5±1.4	—	—	—	—	24.2±0.9	−1.2	24.3±1.2	−0.8	155.7±8.2	535.5
OMC[b]	39.0±8.0	—	—	—	—	88.0±2.1	125.6	170.3±14.2	336.0	33.5±5.5	−14.0
OSC[c]	53.5±9.2	—	—	—	—	76.5±11.4	43.0	85.2±3.8	59.3	146.2±11.9	173.3

a) ECF=enhancement of colony formation.
b) OMC=Ovarian mucinous cystadenocarcinoma.
c) OSC=Serous papillary ovarian carcinoma.
Tumor cell suspension was plated at a concentration of 100,000 (cell lines) or 300,000 (from tumor biopsies) viable tumor cells per plate in the upper layer of the two-layer agar culture system. GM-CSF was tested by addition to the agar matrix to allow continuous exposure during the culture period (average of ten days).
Data from 3 experiments performed at least in triplicate.

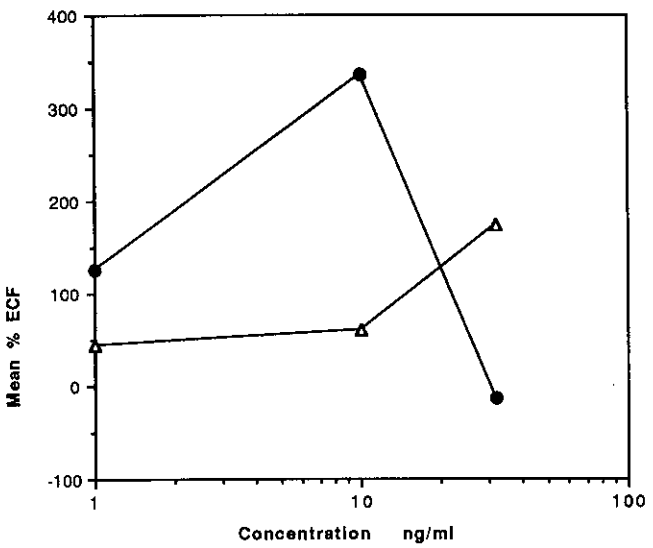

Fig. 2. Dose-response curves for GM-CSF enhancement of colony formation by 300,000 OMC (●) or OSC (△) freshly isolated human ovarian cancer cells.

in cell lines as well as in cells freshly isolated from human specimens. The growth-stimulating effect occurred with concentrations of RhGM-CSF that result in colony formation by normal hematopoietic progenitor cells.[1] It should be emphasized that in IGROV-1, A2774, and PA-1, as well as OMC and OSC, the effect evoked by RhGM-CSF was in the range of concentrations (from 0.1 to 1.0 ng/ml) normally used for bone marrow recovery after chemotherapy.[10,11] Further studies with tumor cells responsive and non-responsive to RhGM-CSF could yield more insight in the biology and growth-controlling mechanisms of neoplasia. From a clinical point of view, it would be important to consider the possible responsiveness of nonhematopoietic tumor cells to RhGM-CSF.

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