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Stimulation of Anchorage-independent Growth of Human Tumor Cells by Interleukin 1

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ABSTRACT

Human peripheral blood monocytes and tumor-associated macrophages release a factor that enhances the clonal growth of a human epithelial tumor cell line (SW-13) in soft agar. We now demonstrate that purified interleukin 1 (IL-1) may account for part of this colony-stimulating activity. Purified IL-1 (0.5 to 8 units/ml) was added to SW-13 cells cultured in soft agar. IL-1 increased colony growth in a dose-dependent manner and did not inhibit colony formation at the highest doses tested. Other purified human monocyte products (α-interferon, tumor necrosis factor, transforming growth factor β, fibronectin) did not stimulate colony growth. Antibody to IL-1 only partially inhibited the ability of monocyte-conditioned medium to stimulate SW-13 colony growth. This antibody did, however, completely inhibit the ability of purified IL-1 to support the growth of SW-13 colonies in soft agar. IL-1 increased growth of quiescent SW-13 cells cultured in monolayers as assessed by tritiated thymidine incorporation assays. The results of this study indicate that IL-1 can enhance clonogenic growth of an epithelial cell line in soft agar. However, other uncharacterized activities in monocyte conditioned medium also promote colony growth. These studies add to an increasing body of evidence indicating that inflammatory products play a role in maintaining the transformed phenotype.

INTRODUCTION

The ability of human tumor cells to form colonies in soft agar has been correlated with tumorigenic potential. The factors controlling the ability of cells to form colonies in soft agar are poorly understood. We previously demonstrated that autologous MO[2] produce factors that enhance anchorage-independent growth of human tumor cells in soft agar. We observed that depletion of phagocytic macrophages from effusions derived from patients with ovarian cancer resulted in a decrease of tumor colonies in soft agar (1). In subsequent studies, we demonstrated that HLA-Dr-negative MO enhanced growth of tumor cells in soft agar and that HLA-Dr-positive cells limited growth (2). We found that irradiation of adherent accessory cells increased their ability to support tumor colony formation. The effect of irradiation was mediated by T-lymphocytes that controlled the ability of MO to produce diffusible tumor colony-stimulating factors (3).

These observations were confirmed by Buick *et al.* (4) using human malignant effusions. Similarly, Welander *et al.* (5) and Saito *et al.* (6) found that cloning efficiencies of primary human ovarian tumor cells were enhanced by both xenogenic and autologous MO. We have recently demonstrated that human peripheral blood MO also secrete factors that enhance growth of clonogenic human tumor cells in soft agar (7). These factor(s) were nondialyzable, relatively heat-stable, and acid-labile proteins.

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[2] The abbreviations used are: MO, monocyte-macrophages; MO-CM, monocyte-conditioned medium; TGF, transforming growth factor; TNF, tumor necrosis factor; FN, fibronectin; IL-1, interleukin 1; IFN, interferon; FBS, fetal bovine serum; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; FGF, fibroblast growth factor.

IL-1, originally defined as a monocyte-derived factor mitogenic for thymocytes, is now known to affect many biological activities. IL-1 serves as an important mediator of the inflammatory response by acting as a growth factor for fibroblasts (8), by inducing release of prostaglandin E_2 (9), and by stimulating the secretion of proteases (10). IL-1 also promotes growth of glial (11), mesangial (12), and endothelial (13) cells and osteoclasts (14). Most recently, IL-1 has been demonstrated to be cytotoxic for certain human tumor cell lines (15–17), but mitogenic for other tumor cell lines (18–20).

As IL-1 is a component of human MO-CM, we examined the effect of highly purified human IL-1 on the ability of a human tumor epithelial cell line (SW-13) to clone in soft agar. We found that purified IL-1 enhanced the anchorage-independent growth of this cell line.

MATERIALS AND METHODS

Cell Culture. The SW-13 cell line (CCL 105), derived from a human adenocarcinoma of the adrenal, was obtained from the American Type Culture Collection (Rockville, MD). The cells were grown at 37°C in L-15 medium (Grand Island Biological Co., Grand Island, NY) supplemented with 10% FBS (Sterile Systems, Logan, UT). All cell lines were used in the logarithmic growth phase for experiments within 25 passages of the original frozen stock.

Soft Agar Colony Assay. Base layers of 1 ml of 0.5% agar (Difco, Detroit, MI) containing enriched McCoy's Medium 5A (21) were prepared in 35-mm plastic Petric dishes. Conditional medium derived from monocytes or IL-1 (see following) were incorporated into the base layers at the concentrations indicated in the text. A 1-ml overlayer of 0.3% agar containing SW-13 cells (1.5×10^4) in enriched Connaught Medical Research Laboratories medium (21) was applied. Plates were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air, and colonies of more than 40 cells were counted between 10 and 14 days after plating in an inverted phase microscope or by a Bausch-Lomb FAS II image analyzer.

Preparation of Monocytes and MO-CM. Human peripheral blood mononuclear cells were obtained by Ficoll-Hypaque density centrifugation (1.077 g/ml). Mononuclear cells were incubated in 100-mm tissue culture dishes (Falcon No. 3003) at a concentration of 2×10^6 cells/ml in RPMI 1640 medium and 10% FBS for 2 h. The nonadherent cells were discarded, and the plates were washed 5 times in cold phosphate-buffered saline. The adherent cells were more than 95% positive for a naphthyl acetate esterase and 90% viable as determined by trypan blue dye exclusion. To prepare CM, monocytes were allowed to remain on dishes and cultured for 3 days at 37°C in 5% CO_2 in air in RPMI 1640 medium supplemented with 10% FBS. The MO-CM was removed from the dishes, filtered, and used fresh or stored at 4°C for future use.

Purified Products and Antibodies. Purified IL-1, derived from human monocytes stimulated with *Staph. albus*, was obtained from Genzyme (Cambridge, MA). The preparation had a specific activity of 8×10^6 units/μg of protein and was free of endotoxin, IL-2, and interferon activity. Human recombinant TNF-α was obtained from Genentech (South San Francisco, CA). Human recombinant IFN-α was obtained from Schering Corp. (Kenilworth, NJ), fibronectin from Collaborative Research (Lexington, MA), and TGF-β from BTI Technologies (Cambridge, MA). Antibody to human monocyte IL-1 (Genzyme) was prepared in rabbits. This preparation neutralized one unit of native or recombinant (pisoelectric points 5 and 7) IL-1 at a 1:1000 dilution.

Thymidine Incorporation Assays. The levels of [^{3}H]thymidine incorporation were determined in quiescent cells by the method of Halper and Moses (22). SW-13 cells were plated at 1×10^4 cells per well in RPMI 1640 medium and 10% FBS in 24-well tissue culture plates (Falcon). Four days after plating, confluent resting cells were stimulated to grow by changing to fresh RPMI 1640 medium containing either 10% FBS or RPMI 1640-10% FBS supplemented with the indicated concentration of interleukin 1. [^{3}H]Thymidine incorporation was determined 24 h after medium changes. Cells were pulsed for 60 min with 1.0 μCi of [*methyl*-^{3}H]thymidine (6.7 μCi/mmol; New England Nuclear, Boston, MA) per ml of medium, and the incorporation into acid-precipitable material was determined as described.

Statistical Analysis. The two-tailed Student *t* test was used on paired samples to compare control to experimental groups. Data are expressed as mean ± SD (4 plates per point). Statistical significance was established at the 5% level.

RESULTS

Effect of Purified Monokines on Growth of SW-13 Cells in Soft Agar. We have previously demonstrated that conditioned medium derived from 3-day-old cultures of human peripheral blood monocytes stimulates anchorage-independent growth of SW-13 cells. We therefore examined whether purified monocyte-derived factors could enhance the ability of SW-13 cells to clone in soft agar. These monokines included purified native human IL-1, tumor necrosis factor, human recombinant α-interferon, human fibronectin, and human platelet-derived TGF-β. Only IL-1 enhanced cloning efficiencies of SW-13 cells at the concentrations tested. The other factors failed to support colony growth when assayed over a wide range of concentrations (Table 1). When SW-13 cells were cloned at higher densities (1×10^5 cells/ml), TNF, fibronectin, and TGF-β at the concentrations indicated in Table 1 did not inhibit colony growth. However, IFN-α inhibited colony growth 50% at 125 units/ml (data not shown).

Growth-stimulatory Effect of Increasing Concentrations of IL-1. IL-1 increased colony growth in a dose-dependent manner until a plateau was reached between 1 and 2 units/ml. Increasing concentrations of IL-1 resulted in slightly but not statistically significant decreased colony growth. The number of colonies observed was always greater than control values observed in the total absence of IL-1 (Fig. 1).

Ability of Antibody to IL-1 to Abrogate the Stimulatory Effect of MO-CM. To determine if all the growth-stimulatory activity of MO-CM was due to IL-1, antibody to IL-1 (at the concentrations indicated) was added to agar cultures containing suboptimal concentrations of MO-CM (0.6 ml/plate). Colony growth was assessed 10 days later. The results (Fig. 2) indicate that increasing concentrations of antibody decreased colony growth stimulated by MO-CM in a dose-dependent fashion. However, even high concentrations of antibody (dilution of 1:64) failed to decrease colony growth more than 50%. This finding suggests that only part of the colony-stimulating activity of MO-CM was due to IL-1. Control, preimmune rabbit serum failed to significantly inhibit colony growth induced by MO-CM. By comparison, varying concentrations of antibody to IL-1 were added to agar cultures of SW-13 cells containing 2 units/ml of IL-1 (Table 2). Antibody at a dilution of 1:500 completely inhibited the ability of IL-1 to increase colony growth. Thus, antibody added to the agar cultures neutralized the growth-stimulatory effect of IL-1. Preimmune rabbit serum failed to inhibit IL-1-induced colony growth.

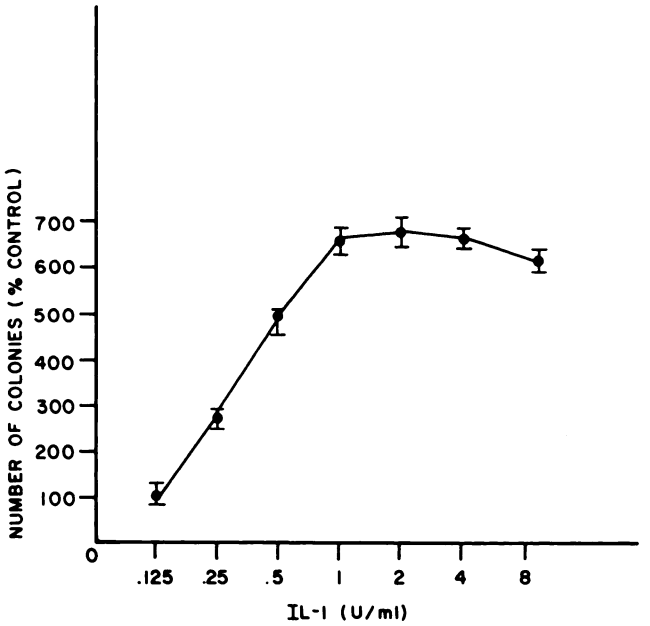

Fig. 1. Effect of IL-1 on growth of SW-13 cells in soft agar. Purified IL-1 at the concentrations indicated was added to cultures of SW-13 cells, and colonies were assessed at Day 10. Results of 5 experiments were normalized to the percentage of control for each experiment. *Points*, mean for each IL-1 concentration; *bars*, SD. The number of control colonies, observed in the absence of IL-1, was 27 ± 6/1.5 × 10^4 cells.

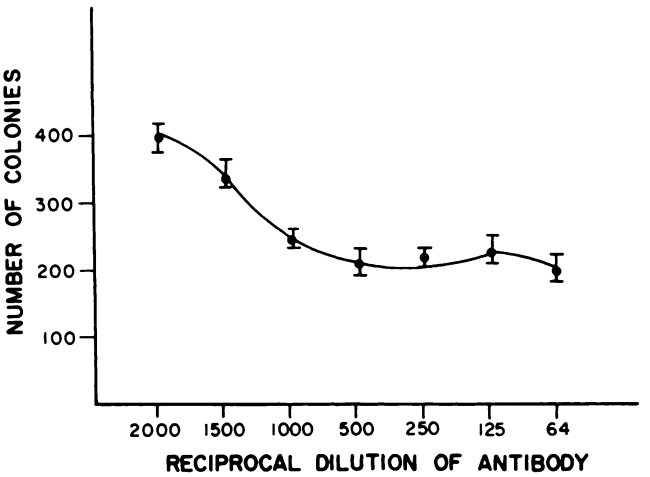

Fig. 2. Effect of antibody to IL-1 on the ability of MO-conditioned medium to stimulate SW-13 growth. MO-conditioned medium (0.6 ml) was added to cultures of SW-13 cells in the presence or absence of the indicated concentrations of antibody to IL-1. Colony formation was assessed at Day 10 as described. The number of control colonies in the absence of IL-1 was 35 ± 6. *Points*, mean; *bars*, SD (4 plates).

Table 1 *Ability of purified monokines to stimulate SW-13 colony growth*

SW-13 cells were cultured at 1.5×10^4 cells/ml in the presence of the indicated concentrations of characterized factors. The ability of these factors to enhance colony growth was assessed as described. The number of colonies observed in the absence of growth factors was 41 ± 25.

Factor	Amount tested/ml	Range of colony enhancement
IL-1, human	0.5–10 units	0.5–12
TNF, human	0.1–10,000 units	0
IFN-α, human	1–10,000 units	0
Fibronectin, human	0–50 ng	0
TGF-β, human (±2.5 ng/ml EGF)	2–5 ng	0

Effect of IL-1 on [^{3}H]Thymidine Incorporation of SW-13 Cells in Monolayer Culture. As growth factors, such as TGF-β, may have opposing effects when assayed in different culture systems, we examined the effect of IL-1 on DNA synthesis of SW-13 cells in monolayer cultures. Quiescent SW-13 cells were stim-

Table 2 *Ability of IL-1 antibody to inhibit IL-1-induced colony formation*

SW-13 cells were cultured as described in the presence of 2 units/ml IL-1 and the indicated concentrations of IL-1 antibody. Colony growth was assessed at Day 10. The number of colonies observed in the absence of IL-1 was 24 ± 12.

Antibody concentration (reciprocal dilution)	No. of colonies/ 1.5×10^4 cells
0	10 ± 10[a]
4	44 ± 40
64	12 ± 0
500	20 ± 18
1000	100 ± 12
Normal rabbit serum (1:4)	200 ± 25
IL-1 alone	290 ± 32

[a] Mean ± SD.

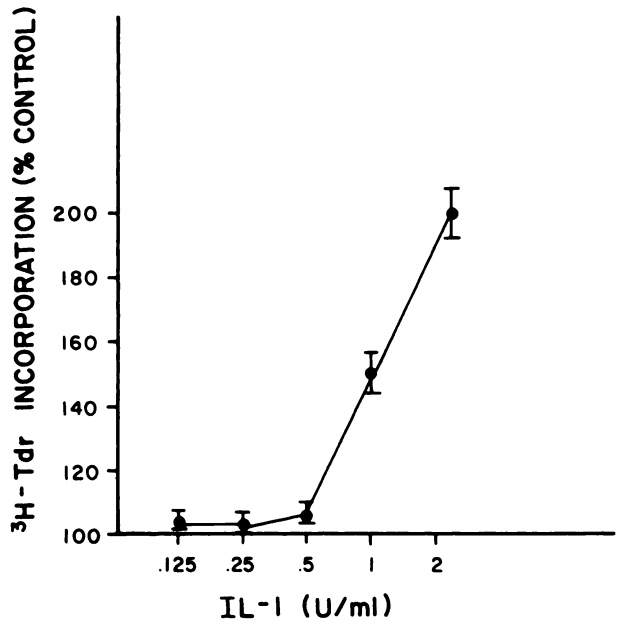

Fig. 3. Stimulation of DNA synthesis in quiescent SW-13 cells. Cells were seeded into 24-mm wells and grown to confluence for 5 days. Cells were then stimulated to synthesize DNA by adding increasing concentrations of IL-1. *Points*, mean of 4 wells; *bars*, SD.

ulated to grow in the presence or absence of varying concentrations of IL-1. IL-1, at concentrations greater than 1 unit/ml, significantly ($P \leq 0.05$) stimulated thymidine incorporation by SW-13 cells in monolayer cultures as compared to unstimulated cells (Fig. 3). Inhibition of cell growth was not observed at any concentration of IL-1 tested.

DISCUSSION

We have demonstrated that MOs derived from human malignant effusions enhance anchorage-independent growth of autologous human tumor cells (1–3). More recently, we observed that a factor or factors secreted by normal human peripheral blood MOs enhance cloning efficiencies of a human tumor epithelial cell line, SW-13, in soft agar (7). We now demonstrate that IL-1, a monocyte-derived growth factor with pleiotropic biological properties, enhances the anchorage-independent growth of this cell line. The growth-stimulating effect of IL-1 was dose dependent, and inhibition of cell growth was not observed at any concentration tested. Work in our laboratory indicates that IL-1 also enhanced growth of a small cell lung carcinoma line (H128) previously shown to be stimulated by MO-CM. IL-1 (2 units/ml) did not enhance or inhibit growth of a human colon carcinoma cell line (Colo 205) or a human breast tumor cell line (MDA-468).[3] Although IL-1 enhances growth of fibroblasts (8, 23), astroglial cells (11), T-lymphocytes (24, 25), and mesangial cells (12), it has only recently been reported to stimulate growth of human tumor cells in either soft agar or monolayer culture (18–20).

Only IL-1 and FGF, of a wide variety of defined growth factors tested either by us or Halper and Moses (22), stimulate anchorage-independent growth of SW-13 cells. This cell line is extremely responsive to concentrations of FGF as low as 50 ng/ml. Normal cultured adrenal cortical cells secrete and are responsive to FGF (26). Cells of this lineage may be controlled by FGF like molecules under physiological circumstances. It is of interest that FGF and IL-1 share growth-promoting activity for several cell lines (21), and a significant amino acid sequence homology exists between basic or acidic FGF and IL-1 (25% and 27%, respectively). The particular amino acid sequences in common may contribute to the biological activity of the molecule. Thomas *et al.* (27) suggested that IL-1 and FGF were members of a homologous family of growth factor proteins different from insulin, platelet-derived growth factor, or epidermal growth factor. The somewhat unique sensitivity of this line to IL-1 may in part be related to its response to FGF.

The other purified monokines tested (IFN, TNF, FN) failed to stimulate growth of SW-13 cells and do not enhance anchorage-independent growth of other human tumor cell lines. Ludwig *et al.* (28) noted, however, an occasional paradoxical stimulation of human tumor cell colony formation in soft agar in the presence of IFN-α. TNF stimulates fibroblast proliferation (29), but has not been reported to enhance growth of tumor cells (30). Fibronectin, an MO product, may mediate the cellular response to TGF-β. Purified FN induces anchorage-independent growth of NRK-49F cells (31). We failed to demonstrate an effect of fibronectin at any concentration tested on SW-13 colony formation. These differences may relate to the fact that SW-13 cells are of epithelial, rather than mesenchymal, original and that high concentrations of FN were not assessed.

Our observations contrast with those of others who demonstrated that IL-1 inhibited *in vitro* growth of several human tumor cell lines. Gaffney and Tsai (16, 32) reported that IL-1 is cytotoxic for human breast tumor cell lines at concentrations greater than 4 units/ml as assayed in monolayer culture. Onazaki *et al.* (15) and Lachman *et al.* (17) found that IL-1 (2 units/ml) inhibits growth of a human melanoma cell line, A 375. In our study, IL-1 at the higher concentrations tested failed to inhibit cell growth. It should be noted that Gaffney and Tsai (16) did find differential sensitivity to IL-1 among human tumor cell lines with breast tumor lines being most sensitive to the inhibitory effects of IL-1.

Addition of antibody to IL-1 to monocyte-conditioned medium failed to completely neutralize the growth-promoting effect of MO-CM. Growth-stimulatory activity was inhibited only 50% by any of a wide range of antibody concentrations. This would suggest that monocyte-conditioned medium contains factors other than IL-1 which enhance colony growth. Similarly, Bitterman *et al.* (33) observed that a variety of alveolar macrophage-derived factors including PDGF, a unique alveolar macrophage-derived growth factor, fibronectin, and IL-1 enhance the proliferation of fibroblasts *in vitro*.

Roberts *et al.* (34) have proposed that transforming growth factors are bipotential. TGFs stimulate anchorage-independent growth, but inhibit anchorage-dependent growth of the same cell lines. We found that IL-1 stimulated both anchorage-independent and -dependent growth of SW-13 cells. Onazaki *et al.* (15) reported that IL-1 inhibited both types of growth of A375 cells. IL-1 does not appear to have opposing biological

[3] Unpublished observations.

effects on growth of adherent or nonadherent cells in these two cell lines.

The ability of monocytes to infiltrate tumors is well characterized, and IL-1, along with other unidentified factors, may enhance tumor growth. Our work indicating that IL-1 stimulates clonogenic growth of human tumor cells adds to a growing body of evidence indicating that products of inflammatory cells may play a role in the maintenance of the transformed phenotype (35).

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