Stimulation of Human Prostatic Carcinoma Cell Growth by Factors Present in Human Bone Marrow

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Abstract

Malignant prostatic carcinoma, a major cause of cancer mortality in males, most often metastasizes to secondary sites in bone. Frequently, the growth rate of the secondary tumor in bone marrow is considerably greater than that of the slowly growing primary prostatic tumor. We now report that two lines of human prostatic carcinoma cells proliferate in response to conditioned media from unstimulated human, rat, or bovine bone marrow. Nonprostatic tumor cell lines showed little or no growth response to the same medium.

The proliferative activity found in bone marrow was not duplicated by any of a variety of purified growth factors including epidermal growth factor (EGF), acidic or basic fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF) alpha or beta, interleukins 1, 2, 3, 4 or 6, granulocyte (G), macrophage (M) or granulocyte-macrophage (GM) colony stimulating factor (CSF). Whereas a mixture of G-CSF, M-CSF, and IL 3 produced a mitogenic response in the prostatic carcinoma cells, these three factors were not present in our bone marrow samples in sufficient quantities to promote the observed proliferative response. To further identify the cellular source of the proliferative activity present in bone marrow–conditioned medium, we tested conditioned media made from human bone marrow stromal cells. The stromal cell conditioned medium stimulated increased growth of the prostatic carcinoma cells to levels equivalent to those observed with the bone marrow conditioned medium. These results suggest that novel mitogenic factors that are produced by bone marrow stromal cells and remain in the bone marrow cavity may account, in part, for the preferential growth of prostatic metastases in bone.

Introduction

Prostatic adenocarcinoma is a slow-growing tumor that afflicts > 50% of the male population over the age of 70 (1). Although only a small percentage of prostatic tumors reach sufficient size to escape from the primary site, metastatic prostate tumors are associated with a high mortality rate and represent a leading cause of cancer deaths among males (2, 3). Prostatic carcinoma metastases are most commonly found in bone marrow, where their growth rate appears to be considerably more rapid than that observed for primary prostatic tumors (4, 5). That certain tissues may provide a preferential environment for the growth of certain tumors was first proposed by Paget in 1889 (6). According to this theory, the osteotropism of prostatic carcinoma cells could result if these cells were particularly responsive to growth factors present in bone marrow.

Bone marrow is the major site of hematopoietic cell growth. It exists as two clearly identifiable components: the hematopoietic cells that make up the majority of the cellular elements and the stromal component that is formed of highly vascular connective tissue (7). The hematopoietic cells are transient in the marrow; upon maturation they move into the blood stream. The stroma, however, remains and serves as a scaffolding upon which the hematopoietic cells can differentiate and mature. The stromal cells in the marrow cavity produce a number of hematopoietic growth factors (HGFs[1]; 8–22); several such colony-stimulating factors (CSFs) have been purified, sequenced, and cloned (23–28). Furthermore, growth factors produced outside the marrow cavity can be sequestered and concentrated in the bone marrow after binding to extracellular matrix elements in the marrow stroma (29).

In this study, we have investigated whether factors present in bone marrow can stimulate the growth of metastatic prostatic carcinoma cells that selectively colonize the bone marrow. Our results demonstrate that bone marrow–conditioned medium (BMCM) stimulates the growth of prostatic carcinoma cells but not of a variety of other tumor cell types. We also show that bone marrow stromal cell–conditioned medium greatly enhances the growth of prostatic carcinoma cells. This activity is not duplicated by any of a variety of known growth factors and may consequently represent the action of a novel mitogenic agent.

Methods

Cell lines. All cell lines were obtained from the American Type Culture Collection, Rockville, MD. PC-3 cells (human prostate adenocarcinoma, derived from bone marrow metastases) were grown in RPMI medium 1640 (Gibco Laboratories, Grand Island, NY) supplemented

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Received for publication 12 April 1988 and in revised form 8 February 1989.

J. Clin. Invest.

0021-9738/89/07/0043/08 $2.00
Volume 84, July 1989, 43–50

1. *Abbreviations used in this paper:* BMCM, bone marrow conditioned medium; CSF, colony-stimulating factor; EGF, epidermal growth factor; FGF, fibroblast growth factor; G, granulocyte; HGF, hematopoietic growth factor; M, macrophage; PDGF, platelet-derived growth factor; TGF, transforming growth factor.

with 10% defined FCS (Hyclone Laboratories, Logan, UT), 0.3 mg/ml glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Irvine Scientific, Santa Ana, CA). Serum was heat-treated for 30 min at 56°C before use. DU 145 cells (human prostate carcinoma, derived from a brain metastasis in a patient with wide-spread metastatic disease) were grown in DME (Gibco Laboratories) supplemented with 10% FCS, glutamine, and antibiotics as were SK-Mel-2 cells (human malignant melanoma, metastatic to skin of thigh), CAKI-1 cells (human kidney carcinoma, metastatic to skin), MCF-7 cells (human breast carcinoma, pleural effusion), Calu-1 cells (human lung epidermoid carcinoma, metastatic to pleura), SK-N-MC cells (human neuroblastoma, metastatic to supra orbital area), and Hs 746t cells (human stomach carcinoma, metastatic to leg). MDA-MB-361 cells (human breast adenocarcinoma, metastatic to brain) were grown in L-15 medium (Gibco Laboratories) with 10% FCS, glutamine, and antibiotics.

Preparation of BMCM. Marrow was obtained by cutting bone fragments with sterile bone clippers and gently scraping out the red or yellow marrow fraction. The bones were then rinsed with 5–10 ml of RPMI 1640 medium that was then added to the marrow. The marrow contents were suspended by gentle pipetting with a 10-ml sterile plastic pipette. The marrow cells were then centrifuged at 700 *g* for 5 min, and resuspended in 1–2 ml of serum-free RPMI medium containing glutamine, penicillin, and streptomycin. The suspension was incubated in 5% CO_2 for 24 h at 37°C. The resulting BMCM was clarified by centrifugation at 800 *g* for 5 min to remove cells and frozen at −20°C. Protein concentrations in the marrow-conditioned media were determined using a protein assay (Bio-Rad Laboratories, Richmond, CA). The protein concentration of the BMCM ranged from 0.5–1.2 mg/ml. For cell growth assays, the conditioned medium was thawed and diluted with sterile RPMI 1640 medium.

BMCM was prepared from human, bovine, and rat bones. Bovine clavicles were kindly furnished by the New Boston Food Market, South Boston, MA. The clavicles were first rinsed with 10% Betadyne solution and marrow was then processed. Rat marrow, obtained by flushing the femurs of 175–200-g Sprague-Dawley male rats (Charles River Breeding Laboratories, Wilmington, MA) with 2 ml of PBS was treated in the same manner described above. Human bone samples, kindly provided by Dr. Robert Shamberger, Children's Hospital of Boston, were obtained from sternal fragments normally removed in the process of correction of pectus excavatum deformity. These bone samples were the source for red marrow. Yellow marrow was obtained from a healthy femur that was amputated as a consequence of peripheral vascular disease. In each case, the collected marrow was suspended in 1 ml of medium and BMCM was obtained as described above.

Bone marrow stromal cell–conditioned medium was kindly provided by Dr. Bing Lim, Beth Israel Hospital, Boston. Bone marrow stromal cells were grown in Iscove's modified Dulbecco's medium (IMDM) (Gibco Laboratories) supplemented with 10% FCS, 10% horse serum (HS), glutamine, and antibiotics to near confluence. The cells were then washed twice with sterile PBS and incubated for 24 h with serum-free IMDM. The conditioned medium was then collected and frozen at −20°C. Protein concentrations were determined using the Bio-Rad protein assay. The protein concentrations ranged from 24 to 38 μg/ml. The stromal cell conditioned medium was centrifuged in centriprep-10 concentrators (Amicon Corp., Danvers, MA).

Preparation of conditioned medium from other human organs. Organ tissue was minced into 1 mm^3 fragments and washed with 5 ml of RPMI 1640 medium. The organ tissue was suspended by gently pipetting with a 5-ml sterile plastic pipet and then centrifuged at 700 *g* for 5 min. The wash medium was discarded and the organ tissue resuspended in 1–2 ml of serum-free RPMI medium containing glutamine, penicillin and streptomycin. The conditioned medium was then prepared as described in the previous section.

All tissue samples were kindly provided by Dr. Mark Litwin, Brigham and Women's Hospital, Boston. Kidney tissue was obtained from a nephral ureterectomy for ureteral carcinoma; skeletal muscle, from a prostatectomy for benign prostatic hyperplasia; and foreskin tissue, from a cosmetic circumcision procedure.

Growth factors. The properties of the recombinant HGFs have been described in detail previously (23–31). All of the hematopoietic factors were generously provided by Dr. Steven C. Clark, Genetics Institute, Cambridge, MA, with the exception of IL 1, 2, and 4, which were from Genzyme Corp., Boston, MA. Recombinant human macrophage-colony stimulating factor (M-CSF) and granulocyte-macrophage-CSF (GM-CSF) were expressed in Chinese hamster ovary (CHO) cells engineered to release high levels of the factors into culture medium. Assays were conducted using M-CSF that was partially purified (~ 50% pure) and GM-CSF that was highly purified (> 95% pure) from the CHO-conditioned medium. The partially purified M-CSF preparations stimulated half of the maximal level of murine macrophage colony formation at a dilution of 1:300,000. Highly purified GM-CSF had a specific activity of $1–4 \times 10^6$ U/mg when assayed for the ability to stimulate proliferation of primary blasts from patients with chronic myelogenous leukemia (CML).

Recombinant G-CSF and IL 3 were expressed in monkey cos cells. Cos cell–conditioned medium containing G-CSF stimulated human granulocyte colony formation with half-maximal activity at a dilution of 1:100,000. Assays for IL 3 activity were conducted using nonfractionated IL 3 containing cos cell–conditioned medium or with IL 3 that had been purified to near homogeneity from this medium. The nonfractionated material stimulated half-maximal proliferation of CML blasts at a dilution of 1:100,000 whereas the purified IL 3 had a specific activity of 2×10^7 U/mg when tested in the same assay. Mock conditioned media from non-transfected CHO cells or cos cells were used as controls. IL 1–6 were tested as cos cell–conditioned medium and displayed half-maximal activity on murine bone marrow colony formation at a dilution of 1:10,000. IL 1 was purified from monocytes that had been stimulated with heat-killed *Staphylococcus albus.* The IL 1 preparations contained 8×10^6 U of activity per μg of protein where one unit is the amount required to double the proliferative response of mouse thymocytes after stimulation with 1 μg/ml of PHA. Transforming growth factor beta (TGF-beta), from fresh human or porcine platelets, was purchased from R & D Systems, Inc., Minneapolis, MN. The half-maximal activities for the stimulation of colony formation in soft agar has been determined to be 1 ng/ml for AKR-2B fibroblasts and 0.1–0.4 ng/ml for normal rat kidney (NRK) (49F) fibroblasts. This preparation was found to be at least 97% pure by NH_2-terminus analysis and analysis on silver-stained gels. Transforming growth factor alpha (TGF-alpha), purchased from Creative BioMolecules, Hopkinton, MA, was expressed in *Escherichia coli* and determined to be 99% pure by reverse phase HPLC. Maximal activity was determined to be ~ 0.05 ng/ml in mouse 3T3 cells. Epidermal growth factor (EGF) and basic fibroblast growth factor (b-FGF) were purchased from AMGen Biologicals, Thousand Oaks, CA. EGF was prepared from an *E. coli* host recombinant DNA procedure and purified to 98% purity by sequential chromatography. The half-maximal specific activity was found to be 0.1 ng/ml in NRK cells. bFGF was prepared by recombinant DNA procedures in *E. coli* and found to be at least 95% pure by sequential chromatography. The half-maximal specific activity, determined by [^{3}H]thymidine uptake by mouse 3T3 fibroblasts, was 20–50 pg/ml. Acidic fibroblast growth factor (a-FGF) was kindly provided by Dr. Patricia D'Amore, Children's Hospital, Boston. a-FGF was purified to a single band on silver-stained gels and confirmed by Western blot analysis. One unit was defined as the half maximal activity, determined by [^{3}H]thymidine incorporation by mouse 3T3 fibroblasts. Purified platelet-derived growth factor (PDGF) was kindly provided by Dr. Harry Antoniades, Center for Blood Research, Boston. The purified PDGF was obtained from clinically outdated human platelets and prepared as described (32). Bacterial LPS (endotoxin) was obtained from Sigma Chemical Co. (St. Louis, MO).

Cell growth experiments. Cell proliferation assays were performed in 24-well tissue culture dishes (Costar Corp., Cambridge, MA). Cells were seeded at initial densities of $5–10 \times 10^3$ cells per well in RPMI 1640 medium containing 1% FCS and allowed to plate overnight. The next day, each well received fresh medium containing 0.5–1% FCS plus varying concentrations of bone marrow or control tissue–condi-

tioned medium, bone marrow stromal cell–conditioned medium or growth factors. Experiments were terminated by trypsinizing the cells and counting the cell number using a Zf particle counter (Coulter Electronics, Hialeah, FL). For experiments running > 5 d, fresh medium and growth factors were added to the wells on day 5.

Results

BMCM stimulates prostatic adenocarcinoma cell growth. To determine whether factors derived from human bone marrow could support the growth of prostatic carcinoma cells, PC-3 human prostatic carcinoma cells were incubated for 4 d in culture medium containing 1% FCS and increasing concentrations of human BMCM. As shown in Fig. 1, increasing concentrations of BMCM effectively stimulated PC-3 cell proliferation in a dose-dependent fashion. Maximal proliferation was seen at a protein concentration of 30 μg/ml with half-maximal activity at 15 μg/ml. As a control, conditioned medium made from other human organs was tested at the same doses. The skin, muscle, and kidney had little or no effect on the growth of the prostatic carcinoma cells. When incubated with BMCM prepared from yellow marrow, the prostatic carcinoma cells showed no significant response (data not shown). The kinetics of cell growth were determined in a separate experiment shown in Fig. 2. After 8 d, cell number had increased from 1.0×10^4 to 1.1×10^5 for PC-3 cells incubated in medium containing 0.5% FCS plus 65 μg/ml of BMCM, whereas cells cultured under the same conditions but without BMCM increased to only 2.75×10^4 cells per well.

Differential response of various tumor cell types to bone marrow conditioned medium. The human BMCM did not stimulate proliferation of all tumor cell types. As shown in Fig. 3, the proliferative activity was greatest for two human cell lines, PC-3 and DU 145 derived from prostatic carcinoma metastases (33, 34). These cell lines showed an increase over background of 124 and 96%, respectively. Cell lines derived from patients with other types of metastatic tumors showed significantly reduced responsiveness to BMCM-derived factors. No response was seen with CAKI-1 (human kidney carcinoma, metastatic to skin) or Calu 1 (human lung epidermoid carcinoma, metastatic to pleura) cells. SK-Mel-2 melanoma (human malignant melanoma, metastatic to skin of the thigh) and MCF-7 (human breast carcinoma, pleural effusion) cells

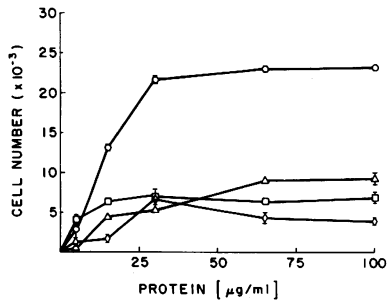

Figure 1. Stimulation of PC-3 prostatic carcinoma cell growth by human BMCM and other human organ conditioned medium. PC-3 cells were plated at a density of 10^4 cells per 16-mm tissue culture well in 1.0% FCS. After 18 h, BMCM (○), kidney-CM (△), muscle-CM (□), and skin-CM (◇) was added to the cultures at the indicated concentrations. After an additional 4 d, the cells in each well were trypsinized and counted. Data are expressed as the mean±SD (n = 3) for the increase in cell number over a background increase of 1.7×10^4 cells. As a positive control, cells incubated in 5% serum grew to a cell density of 4×10^4 over the same time period.

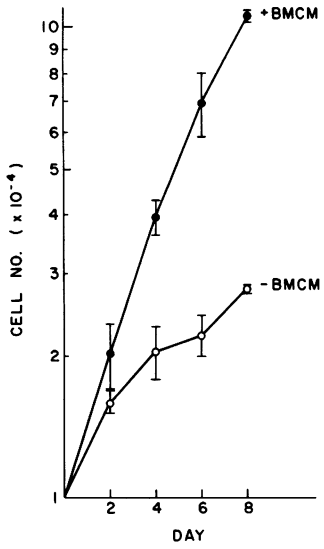

Figure 2. Growth kinetics of PC-3 cells grown in the presence or absence of human BMCM. Cells were plated as described in the legend to Fig. 1, and incubated in control medium in the presence (●) or absence (○) in medium supplemented with 65 μg/ml BMCM. The cells were trypsinized and counted on the days indicated. Data are expressed as the total number of cells per well (mean±SD, n = 3).

showed a significantly smaller response (20 and 37% increase, respectively) than that observed with either of the prostatic cell lines. Cell lines that showed a modest response relative to the prostatic cell lines included the MDA-MB-361 (human breast adenocarcinoma cells, metastatic to brain, 40%), Sk-N-MC (human neuroblastoma, metastatic to supra-orbital area, 36%), and Hs 746T (human stomach carcinoma, metastatic to leg, 38%). Whereas the background growth of the different cell types in 1.0% calf serum varied considerably, the two prostatic tumor cell lines represent both extremes of the spectrum, with PC-3 cells displaying slow background growth and DU 145 displaying rapid background growth; yet, both cell types showed markedly increased growth in the presence of the marrow-conditioned medium. Although no individual cultured cell line can be considered completely representative of its in vivo counterpart, the results described above strongly suggest that prostatic carcinoma cells are more highly respon-

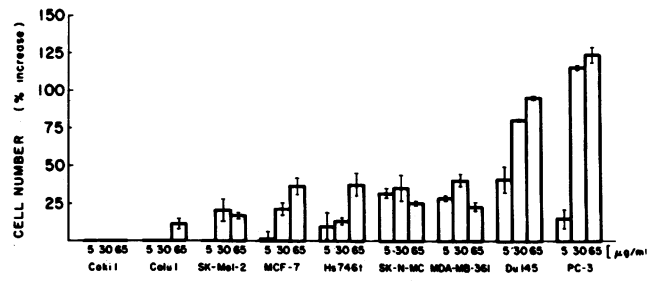

Figure 3. Growth of human tumor cells in response to human bone marrow-conditioned medium. Cells were plated as described in the legend to Fig. 1. BMCM was added after 18 h at the indicated concentrations. After 5 d, the cells in each well were trypsinized and counted. Data are expressed as the mean±SD (n = 3) for the percent increase in cell number over background. Cell lines tested included CaKi kidney carcinoma (7.0×10^4), Calu 1 lung carcinoma (2.5×10^4), SK-Mel-2 melanoma (2.6×10^4), MCF-7 breast carcinoma (3.7×10^4), Hs746T stomach carcinoma (1.2×10^4), SK-N-MC neuroblastoma (1.1×10^4), MDA-MB-361 mammary adenocarcinoma (1.2×10^4), PC-3 prostatic carcinoma (1.9×10^4), and DU 145 prostatic carcinoma (4.7×10^4 in 0.5% FCS). Numbers in parentheses refer to the control in cell number for each cell type in the presence of 1.0% calf serum only.

sive to bone marrow–derived growth factors than any of the other types of metastatic tumor cells tested.

Effect of growth factors on the proliferation of prostatic carcinoma cells. Bone marrow is the major site of proliferation of hematopoietic cells and mesenchymal cells in the marrow cavity have been shown to produce a variety of HGFs (8, 30, 36). We therefore investigated whether any of the known HGFs could be responsible for the proliferative stimulation of prostatic carcinoma cells. Six human recombinant HGFs were tested alone and in a variety of combinations to determine if they had possessed any mitogenic activity for PC-3 cells. These included IL 1, IL 2, IL 3, IL 4, IL 6, G-CSF, M-CSF, and GM-CSF. When tested alone (Table I), none of the factors demonstrated any substantial proliferative activity on the prostatic carcinoma cells. A modest but significant increase in cell number was noted when M-CSF was added to the culture medium. This increase is, however, only 15% of that observed with BMCM. Endotoxin, a potential contaminant of the bone marrow conditioned medium could account for < 5% of the proliferative activity present in BMCM.

We also tested a number of nonhematopoietic growth factors to see whether they could stimulate the prostatic carcinoma cells as well as the BMCM. As shown previously (32–35), neither EGF, basic or acidic FGF, PDGF, or insulin had any effect on the growth of prostatic carcinoma cells. TGF-beta has been shown to be stimulatory for cells of mesen-

Table I. Effect of Recombinant Human Hematopoietic Factors on the Growth of PC-3 Prostatic Carcinoma Cells

Factor	Concentration	Cell no. increase above background	Factor	Concentration	Cell no. increase above background
	U/ml			*ng/ml*	
IL 1	0.1	0	EGF	0.01	230±457
	1.0	3,120±1,599		0.1	0
	10	1,620±576		1.0	0
IL 2	0.1	2,725±857			
	1.0	5,762±664		*U/ml*	
	10	4,365±567	aFGF	0.1	0
				1.0	0
	μg/ml			10.0	0
IL 3	0.01	0			
	0.1	0		*ng/ml*	
	1.0	0	bFGF	0.001	1,610±868
				0.01	2,230±710
	U/ml			0.1	2,210±712
IL 4	10	400±267	PDGF	0.01	296±337
	100	1,246±302		0.1	1,763±157
	1000	2,218±712		1.0	1,173±305
			TGF-alpha	0.01	418±286
	vol/vol			0.1	145±278
IL 6	0.01%	0		1.0	180±39
	0.1%	0	TGF-beta	0.01	−1,083±160
	1.0%	0		0.1	−2,436±59
M-CSF	0.01%	944±145		1.0	−2,698±17
	0.1%	8,640±1,220			
	1.0%	9,420±1,322		*μg/ml*	
G-CSF	0.01%	860±250	Insulin	0.01	237±335
	0.1%	425±350		0.1	712±253
	1.0%	599±200		1.0	902±30
			Endotoxin	0.01	2,380±680
	ng/ml			0.1	3,080±1,080
GM-CSF	0.1	0		1.0	0
	1.0	0			
	10	0	BMCM	100	65,300±4,730

PC-3 cells were seeded at an initial density of 5×10^3 cells per 16-mm tissue culture well in medium containing 1% serum. After 24 h, growth factors were added in fresh medium containing 0.5% serum. Fresh medium with growth factors was added on day 5 and the cells per well were counted on day 10. Growth factor concentrations are expressed in units of activity, gram/milliliter, or as the dilution of conditioned medium from cos or CHO cells that express each factor as described in Methods. All data represent the mean of three experimental points±SEM. Background growth of PC-3 cells over the same time period in the absence of growth factors was 8.0×10^3.

chymal origin and inhibitory for cells of epithelial origin. Prostatic carcinoma cells are of epithelial origin (33) and TGF-beta proved to be inhibitory. TGF-alpha is known to be secreted by a number of human tumors. When we incubated the prostatic carcinoma cells with TGF-alpha, we found that it had no stimulatory effect on these cells.

Recent evidence has demonstrated that combinations of HGFs have a synergistic effect on the growth of hematopoietic precursors (31, 38–44). To determine whether combinations of hematopoietic factors would promote an increased stimulation of prostatic carcinoma cell growth, we incubated PC-3 cells with mixtures of two or more factors. No dramatic increase in cell growth was seen when G-CSF, M-CSF, GM-CSF, and IL 3 were combined in all possible combinations of two factors (Fig. 4). However, when combinations of three factors were tested, the combination of G-CSF, IL 3, and M-CSF produced a significant increase in cell growth. The addition of IL 1, IL 2, IL 3, IL 4, IL 6, or GM-CSF to this combination did not increase the proliferative response (data not shown). Mock conditioned media from nontransfected CHO or cos cells did not contain significant proliferative activity for PC-3 cells when tested in appropriate dilutions and combinations. The above results demonstrate that combinations of HGFs display proliferative activity for prostatic carcinoma cells. Note, however, that the increase in cell growth observed with the combination of three HGFs is < 30% of that observed when cells are incubated with BMCM for the same time period. In addition, bioassays demonstrate that these three factors are not present in significant levels in our BMCM (data not shown). Thus while these experiments show that it is possible for HGFs to act in combination to promote nonhematopoietic cell growth, the hematopoietic factors tested are not responsible for the rapid growth of prostatic carcinoma cells in bone marrow.

Effects of bone marrow stromal cell–conditioned medium. Data obtained from in vitro studies of long-term bone marrow cultures have shown that stromal cell conditioned medium contains diffusible factors that can modify the proliferative activity of stem cells (21). We therefore tested human bone marrow stromal cell–conditioned medium for growth-promoting activity on the prostatic carcinoma cells. Our results, shown in Table II, demonstrate that cloned human marrow stromal cells release growth factors with significant mitogenic activity for prostatic carcinoma cells equivalent to that found in crude BMCM. This result implies that the predominant mitogenic activity in BMCM is contributed by the stromal cell population.

Comparative mitogenic activity of BMCM derived from different species and different bone sites. To determine whether the marrow-derived activity was species specific, we prepared BMCM from bovine clavicles and from rat femurs and compared the mitogenic activity of these factors with that of the human sternal marrow. As shown in Fig. 5, growth-stimulating activity for PC-3 cells was found in all three marrow preparations with greatest activity seen in the bovine material. These results demonstrate that the ability to support PC-3 cell proliferation is a property of bone marrow from a variety of species as well as from different bone sites.

Discussion

It has long been appreciated that certain human tumors metastasize preferentially to particular organs (6). The reasons for such specificity are many and include: (*a*) mechanical factors such as proximity or ease of access of tumor cells to a particular site (45); (*b*) adhesive factors that facilitate tumor cell arrest in a particular tissue (46); (*c*) chemotactic factors that facilitate passage of cells across vascular walls and into particular tissues (47, 48); and (*d*) growth factors or inhibitors that modulate the growth of tumor cell populations in particular tissues (49–51). It is now well established that these mechanisms are not mutually exclusive and that the successful colonization of a given organ by a given tumor cell type will generally involve a com-

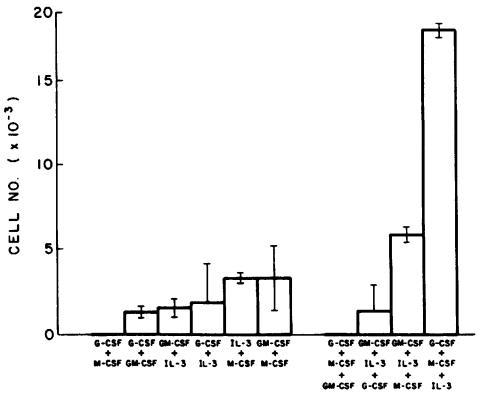

Figure 4. Growth of PC-3 prostatic carcinoma cells in response to combinations of recombinant hematopoietic growth factors. Cells were plated at 5×10^3 cells per 16-mm well. After 18 h, growth factors were added to the wells at the following concentrations: IL 3 (200 ng/ml), GM-CSF (10 ng/ml), G-CSF (0.2%), and M-CSF (0.1%). After 5 d, the cells received fresh medium containing growth factors and after 10 d the cells were trypsinized and counted. Data are expressed as the mean±SD (n = 3) for the increase in cell number over a background increase of 1.2×10^4 cells.

Table II. Effect of Human BMCM and Bone Marrow Stromal Cell–Conditioned Medium on PC-3 Prostatic Carcinoma Cells

Factor	Concentration	Cell no. increase above background
	μg/ml	
BMCM	5	2,800±1,129
	15	13,200±300
	30	21,700±471
	65	23,100±89
	100	23,350±61
BMS-CM	5	1,420±529
	15	10,475±702
	30	16,827±535
	65	26,283±225
	100	31,570±228

PC-3 cells were seeded at an initial density of 1.0×10^4 cells per 16 mm tissue-culture well in medium containing 1% serum. After 24 h, BMCM and BM stromal cell CM was added in fresh medium containing 1% serum. On day 4 the cells in each well were trypsinized and counted. BMS-CM, bone marrow stromal cell–conditioned medium. Conditioned medium concentrations are expressed as micrograms/milliliter of protein. All data represent the mean of three experimental points + SEM. Background growth of PC-3 cells over the same time period in the absence of conditioned medium was 1.1×10^4.

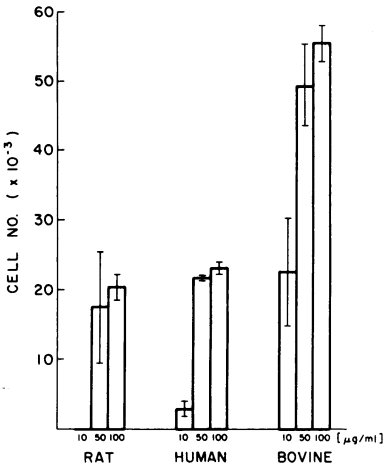

Figure 5. Growth of PC-3 prostate carcinoma cells in response to growth factors derived from rat, human, or bovine bone marrow. Cells were plated at 10^4 cells per culture well and incubated for 4 d in the presence of increasing concentration of marrow-conditioned medium prepared from rat femur, bovine clavicle, or human sternum. Each preparation has the ability to stimulate growth of the prostatic carcinoma cells. Data are expressed as the mean±SD (n = 3) for the increase in cell number over a background increase of 1.4×10^4.

bination of adhesive, chemotactic, and mitogenic stimuli (52–56).

Prostatic carcinomas most frequently metastasize to cancellous bone, with greatest frequency to central sites such as the spine, pelvis, femur, and ribs (4, 5). It has long been thought that this process is facilitated by the existence of specialized channels first described by Batson (57, 58) that run between the prostate and the spine. The possibility that controls on cell growth might also influence the pattern of prostatic cancer metastasis is suggested by the fact that primary prostatic tumors grow extremely slowly over the course of 30–40 yr before exiting the primary site, yet metastatic prostate tumors grow rapidly in secondary bone sites. Thus, although the ratio of metastatic to nonmetastatic prostatic tumors is quite low (< 5%), the metastatic tumors that do become established grow rapidly and account for a significant mortality among older males (3).

It has been suggested that products of resorbing bone can stimulate growth and chemotaxis of bone-seeking tumors (59, 60). This may apply to tumors such as the Walker rat carcinoma, human neuroblastomas, and human mammary carcinomas that cause lytic bone degradation at the site of secondary tumor growth. Prostatic tumors, on the other hand, rarely cause osteolysis and are more frequently associated with osteosclerosis or the build-up of bone mass (5). For such osteosclerotic tumors, the presence of bone resorption products should be minimal and consequently, other factors in bone marrow might be expected to play a more direct role in the establishment of metastases to bone. We therefore tested products of human, rat, and bovine bone marrow for their ability to stimulate the proliferation of various human tumor lines. Our results demonstrate that bone marrow–derived factors markedly stimulate the proliferation of two human cell lines derived from prostatic cancer metastases, the PC-3 and DU-145 cell lines. Cell lines derived from other human tumors with differing metastatic preferences showed a significantly lower response to the bone marrow–derived mitogens. These results demonstrate the presence of mitogenic factors for prostatic carcinoma cells in normal bone marrow and suggest that such factors may influence the enhanced growth rate displayed by prostatic carcinoma cells that have metastasized to secondary bone sites.

Because bone marrow is the major site of hematopoiesis, we inquired whether any of the known HGFs not only had the capacity to stimulate hematopoietic cell proliferation but also resulted in increased growth of human prostatic carcinoma cells. No dramatic proliferative activity for PC-3 cells was noted when IL 1, IL 2, IL 3, IL 4, IL 6, G-CSF, M-CSF, or GM-CSF were tested alone or in combinations of two factors. Only the addition of the three HGFs M-CSF, G-CSF, and IL 3 caused an increase in PC-3 cell growth. Although it is of interest that combinations of HGFs stimulate prostatic carcinoma cell growth, these particular factors cannot be responsible for the mitogenic activity observed in BMCM for the following reasons: (*a*) the proliferative response seen with this combination of HGFs was considerably lower than that observed with the BMCM; (*b*) significant amounts of M-CSF, G-CSF, and IL-3 were not detected by bioassay of our BMCM; and (*c*) activity for human prostatic carcinoma cells was found in rat BMCM even though rodent IL 3 has no activity on human cells (24). Thus, although our results demonstrate that some combinations of HGFs can stimulate prostatic carcinoma cell growth, they do not imply that these particular factors are responsible for the activity observed in BMCM.

A number of nonhematopoietic growth factors have been shown to have growth promoting activity on cells of epithelial or mesenchymal origin. These factors include EGF, acidic and basic FGF, PDGF, and insulin (61). When we tested these factors on the human prostatic carcinoma cells, we found that they had no significant mitogenic effect. Transforming growth factors are polypeptides that promote phenotypic transformation of normal mammalian cells; TGF-alpha is a potent mitogen, whereas TGF-beta is inhibitory for epithelial cells. In our experiments, TGF-alpha had no stimulatory effect on the prostatic carcinoma cells and TGF-beta proved to be inhibitory.

Little is known about the nature of the interaction between hematopoietic cells and their supporting stroma. It is not clear whether the stromal cells are merely acting as a supportive matrix providing a privileged site for hematopoiesis to occur or whether the stromal cells themselves play a role in determining cell lineage development within the hematopoietic system (22). Studies of long-term bone marrow cultures have shown that stromal cell conditioned medium contains diffusable factors that can inhibit or stimulate DNA synthesis of stem cells (21). Our results show that the prostatic carcinoma cells are highly responsive to a growth factor secreted by the bone marrow stromal cells. The identification of the agent responsible for this potent proliferative activity in BMCM remains to be determined.

The finding that human and other mammalian bone marrows contain mitogenic factors for prostatic carcinoma cells may provide new insight into our understanding of the dissemination and growth of metastatic prostatic tumors. This observation may be useful for devising strategies to identify primary prostatic tumors containing sizable populations of cells that are responsive to the marrow-derived factors. Such primary tumors would be at increased risk for metastasis and therefore be prime candidates for surgical removal.

Acknowledgments

We thank Dr. Steven Clark for generously providing the recombinant HGFs used in this study and for performing bioassays on the bone

marrow preparations. Bone marrow stromal cell–conditioned medium was kindly provided by Dr. Bing Lim. This work was supported by grant CA-37393 from the National Cancer Institute and by a grant from the Deutsche Forschungsgemeinschaft to C. Niemeyer. M. Chackal-Roy is an AT&T/Bell Laboratories predoctoral fellow.

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