Partial Characterization of a Bone-Derived Chemotactic Factor for Tumor Cells

F. William Orr, MD, James Varani, PhD, Monica D. Gondek, MS, Peter A. Ward, MD, and Gregory R. Mundy, MD

Medium that has been bathing organ cultures of resorbing bone contains a factor that is chemotactic for cultured Walker carcinosarcoma cells, as assayed by the Boyden chamber technique. There is a positive correlation between the chemotactic activity released by the resorbing bones and the extent of resorption as measured by release of previously incorporated ^{45}Ca from the bones. Generation of the chemotactic factor occurs independent of the humoral mediator of bone resorption. The tumor cell chemotactic factor has a steep dose-response curve, with a fall from maximal to minimal activity extending over a fourfold dilution. The chemotactic activity is stable to heating and has an estimated molecular weight of 6000 daltons, as determined by gel filtration chromatography and retention of activity following dialysis. The chemotactic activity has been distinguished from the tumor cell chemotactic factor derived from the fifth component of complement because the former is not inactivated by antiserum to C5 and because it is chemotactic for EL-4 lymphoma cells, whereas the latter is not chemotactically active for these cells. (Am J Pathol 1980, 99:43-52)

MOST DEATHS from cancer are related to metastatic disease rather than the primary tumor. While the mechanisms involved in tumor metastases are complex,[1,2] it is likely that knowledge of tumor cell motility and its control is important for our understanding of this process.[3] It has been shown recently that the migration of tumor cells can be directed by chemical stimuli [4,5] in a manner analogous to the mechanism by which chemotactic factors direct the motility of leukocytes. We have shown that neoplastic cells are capable of chemotactic responses to a fragmentation product of the fifth component of complement.[5-9] More recently, we have demonstrated that chemotactic stimuli are released from bone during the physiologic process of bone resorption.[10] These chemotactic stimuli cause directed migration of cells derived from the Walker carcinosarcoma, a malignant tumor having a propensity to cause hypercalcemia and to me-

From the Department of Pathology and Department of Medicine, University of Connecticut, Health Center, Farmington, Connecticut, and the Department of Pathology, University of Manitoba, Winnipeg, Manitoba, Canada.

Supported by grants from the Medical Research Council of Canada, the National Cancer Institute of Canada, and by Grants AI-09651, CA-25500 and AM-21584 from the National Institutes of Health. Dr. Orr is the recipient of a career award from the National Cancer Institute of Canada. Dr. Mundy is the recipient of Faculty Research Award FRA-148 from the American Cancer Society.

Accepted for publication November 1, 1979.

Address reprint requests to F. William Orr, MD, Department of Pathology, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, Canada, R3E 0W3.

0002-9440/80/0407-0043$01.00

© American Association of Pathologists

tastasize to bone.[11,12] Ozaki et al have provided experimental evidence that the responses of circulating tumor cells to chemotactic stimuli may be responsible for their transvascular migration and thus a means by which they are able to establish secondary extravascular metastases.[13] These data, in conjunction with our observations on the bone-derived factor, suggest that not only transvascular migration but also selection of a secondary site may be components of the metastatic process that are influenced by chemotactic responses. This report is concerned with the preliminary characterization of the bone-derived chemotactic factor for tumor cells. The generation of the factor, its size, and its ability to elicit chemotactic responses by two different tumor cell types will be shown.

Materials and Methods

Source of Bone-Derived Factor

The bone-derived chemotactic factor used in these experiments was obtained from the conditioned medium harvested from organ cultures of resorbing fetal rodent long bones. The technique of organ culture of long bones has been discussed previously.[14,15] Rats or mice were given subcutaneous injections of .04 mCi of ^{45}Ca on the eighteenth or sixteenth days of gestation, respectively. The following day the fetuses were removed, and the tubular mineralized shafts of the radius and ulna were dissected from the cartilaginous ends and surrounding soft tissue. These explanted shafts were then cultured for 24 hours in control medium to allow for exchange of loosely complexed ^{45}Ca and stable calcium in the medium. The bones were then cultured for 72–96 hours with known stimulators of bone resorption such as parathyroid hormone, prostaglandins, or the bone resorbing factor produced by cultured Walker carcinosarcoma cells.[10] After this period of culture the medium bathing the long bones, which contained the degradation products of bone resorption, was harvested and stored at −20 C for evaluation in the chemotaxis assay. The extent of long bone resorption was measured by the release of total radioactivity from individual bones into the medium during the period of culture. This was compared with calcium release from bones cultured in control medium. Up to 20 bones were used in each treatment group, and statistical differences between the means were determined using the Student *t* test for unpaired samples.

Preparation of Chemotactic Factors from C5

The fifth component of complement (C5) was isolated from human serum by the method of Nilsson et al.[16] The purity of the C5 preparation was tested by immunodiffusion, using goat anti-human C3, goat anti-human C5, and goat anti-normal human serum. The C5 preparation formed a single precipitation band with anti-C5 and with anti-normal human serum. The C5 preparation did not form a precipitation band with anti-C3. The homogeneity of the preparation was also confirmed by electrophoresis in 4% polyacrylamide gels (Biorad Laboratories, Richmond, Calif) in the presence of 0.1% SDS buffer. The leukotactic fractions of zymosan-activated serum were prepared from rat serum by the method described previously.[7] Chemotactic activity for tumor cells was generated by trypsinization of purified C5 or leukotactic fractions of zymosan-activated serum with 1% trypsin w/w at pH 7.2–7.4 for 60 minutes at 37 C. The digestion was stopped by the addition of 2% w/w soybean trypsin inhibitor.[7]

Heat Inactivation and Dialysis of Chemotactic Factors

Aliquots of resorbed bone medium were heated at 56 C for 1 hour before being placed in the lower compartment of Boyden chambers. Other aliquots of the same medium were dialyzed for 24 hours against 50 volumes of control culture medium. For the dialysis experiments membranes were used having stated molecular exclusion sizes of 12,000 daltons (Fisher Scientific Co, Pittsburgh, Pa) and 3500 daltons (Spectrapor #3, Spectrum Medical Industries, Los Angeles, Calif). Chemotactic activity in the heated and dialyzed media was compared to that in untreated medium.

Estimation of Molecular Weight of the Chemotactic Factor by Gel Filtration

Thirty milliliters of medium from cultured resorbing bones were concentrated to a volume of 1.5 ml by ultrafiltration through a hydrophilic membrane having a nominal molecular exclusion size of 1000 daltons (Amicon Diaflo Membrane UM2, Amicon Corporation, Lexington, Mass). Two hundred and fifty microliters of this material were applied to a 25 cm × 1.0 cm column of Sephadex G50 Fine (Pharmacia Fine Chemicals, Piscataway, NJ) that had been equilibrated with 0.05 M phosphate-buffered 0.9% NaCl solution, pH 7.4. The column was calibrated with chymotrypsinogen A (mol wt 25,000), cytochrome C (mol wt 12,500)and insulin (mol wt 6000). Ten fractions of 1.5 ml were collected. Aliquots of each fraction were then tested for chemotactic activity.

Antibody Blocking Studies

Antibodies to C5 were raised in goats and were characterized as previously described.[17] In experiments designed to test the suppressive effect of antibody on preformed chemotactic activity, 25-μl volumes of goat serum containing anti-C5 or 25 μl of normal goat serum (control) were incubated at 37 C for 30 minutes with 1-ml volumes of chemotactic factors derived from C5, from leukotactic fractions of zymosan-activated rat serum, or from cultured bone. These chemotactic factors were then assayed in Boyden chambers.

Cultured Tumor Cells

The tumor cells employed in these studies were the Walker carcinosarcoma and the EL-4 lymphoma. Both cell lines were maintained by serial passage in tissue culture. The techniques for maintaining these cells and for preparing the cells for chemotaxis studies have been described in detail previously.[7]

Chemotaxis Assays

These assays were performed in modified Boyden chambers as described in previous publications.[7,10] For studying the chemotactic responses of tumor cells, micropore filters with a porosity of 12 μ (Schleicher and Schuell, Keene, NH) were used. One milliliter of test medium that was to be assessed for chemotactic activity was placed in the lower compartment. The same culture medium used to suspend the cells was employed as a diluent for the chemotactic factor. The upper compartments were filled with 1.0 ml of tumor cells suspended in a concentration of 5×10^5 cells/ml in culture medium supplemented with 5% fetal calf serum, 50 units/ml penicillin, and 50 μg/ml streptomycin. After 4 hours of incubation at 37 C in 5% CO_2 and air, the filters were removed, fixed, and stained with hematoxylin. The cells that had migrated into the filter to a depth of 15 μ were counted by light microscopy. The experiments were performed in triplicate, and 5 high-power microscopic fields (×400) were counted in each membrane. The mean number of cells/high-power field that had migrated into the filters was determined from the resultant 15 counts.

Table 1—The Generation of Chemotactic Activity for Tumor Cells in Cultures of Bone

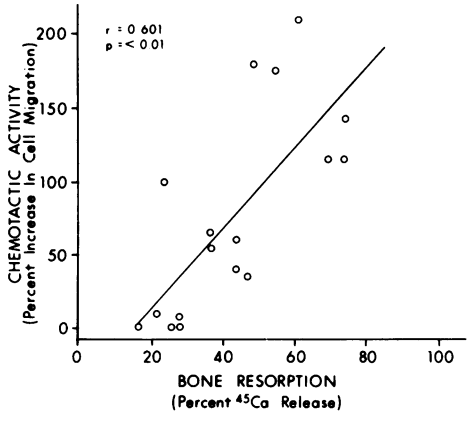
Chemoattractant	Bone resorption (% ^{45}Ca release)		Chemotactic activity (No. cells/HPF ± SE)	
	Experiment A	Experiment B	Experiment A	Experiment B
Mediums from bones induced to resorb*	*Experiment A* 46 ± 2‡	*Experiment B* 61 ± 2‡	*Experiment A* 60 ± 2‡	*Experiment B* 55 ± 3‡
Mediums from control bone cultures†	27 ± 1	24 ± 2	38 ± 1	42 ± 6
Trypsinized C5 (75 μg/ml)	—	—	48 ± 2‡	—
Culture medium only	—	—	31 ± 2	35 ± 3

* Bones cultured in conditioned mediums from Walker carcinosarcoma cultures.
† Bones cultured in unconditioned medium.
‡ Statistically greater than values for control bone cultures, $P < 0.001$.

Results

Generation of Chemotactic Factors

As we have shown before, we found that the addition of supernatants of Walker carcinosarcoma cells to mediums bathing cultured bones stimulated bone resorption as measured by release of the previously incorporated ^{45}Ca from the bones compared with the release of ^{45}Ca from bones cultured in control mediums. When the various mediums were tested for their ability to stimulate the migration of tumor cells into the micropore membranes chemotactic activity was identified in those mediums in which bone resorption had been stimulated (Table 1). We have shown previously that the generation of chemotactic activity for tumor cells in organ culture of resorbing bone is independent of the mediator of bone resorption.[10] In the course of conducting several such experiments in which parathyroid hormone, prostaglandin, and/or Walker cell supernatants



TEXT-FIGURE 1—Correlation of bone resorption and the generation of chemotactic activity for tumor cells. In order to be able to include data from several different experiments, the chemotactic responses are plotted as percent increase in migration compared to nonstimulatory mediums. As indicated in the text, various mediators of bone resorption were employed.

were used as bone resorbing agents, there appeared to be a direct association between the extent of bone resorption (measured by percent of total ^{45}Ca released from cultured bones) and the amount of chemotactic activity contained in the medium (measured by the number of cells migrating into the filter of the Boyden chamber toward the medium from the resorbing bones, compared with control medium). Each of the mediators that stimulated bone resorption generated chemotactic activity in the bone culture mediums. Text-figure 1 is a scatter diagram indicating the correlation in 17 observations between the percent increase in cell migration induced by resorbed bone medium and the percentage of ^{45}Ca released into each of these mediums. The correlation coefficient r between these observations is 0.601 ($P < 0.01$).

Physical Properties of the Bone-Derived Chemotactic Activity

The chemotactic activity present in mediums from resorbing bones was largely stable to heating at 56 C for 60 minutes (Table 2). In two separate experiments, 35% of the original activity was lost after such treatment. All activity was lost when the mediums were dialyzed across a membrane with a putative molecular exclusion size of 12,000 daltons while in two experiments 27% and 30% of the activity was lost when the putative molecular exclusion size of the membrane was 3500 daltons. The behavior of the chemotactic activity on Sephadex G50 gel filtration chromatography was consistent with the above dialysis data. The greatest activity eluted from the column with proteins of molecular weight about 6000 daltons (Text-figure 2).

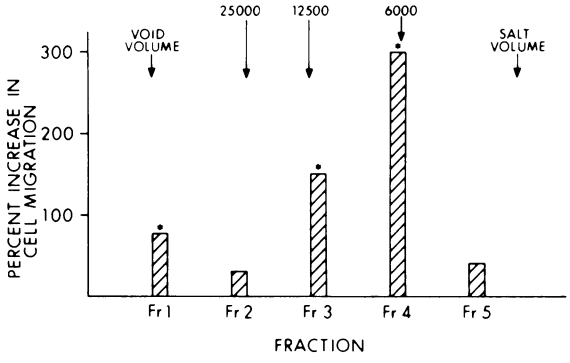
Effect of Incubation with Anti-C5

These experiments (Table 3) were done in order to examine the possibility that the bone-derived chemotactic factor is related to the factor that can be generated by trypsinization of C5 or partially purified fractions of zymosan-activated serum.[7] Medium from bone cultures and medi-

Table 2—Effects of Temperature and Dialysis on the Chemotactic Activity for Tumor Cells Present in Supernatants From Resorbing Bone

Chemotactic sample	Cell migration/HPF ± SE	
	Experiment A	Experiment B
Bone-derived factor	48 ± 2*	62 ± 3*
Bone-derived factor, heated (56 C, 60 min)	41 ± 2*	53 ± 2*
Bone-derived factor, dialyzed (cut off 3500)	42 ± 2*	55 ± 2*
Bone-derived factor, dialyzed (cut off 12,000)	25 ± 2	34 ± 3
Control medium	28 ± 1	36 ± 2

* Significantly greater than corresponding control, $P < 0.05$.



TEXT-FIGURE 2—Gel filtration on G50 Sephadex of concentrated resorbed bone mediums. Fractions were assayed for chemotactic activity for tumor cells. The column was calibrated with chymotrypsinogen A (mol wt 25,000), cytochrome C (mol wt 12,500), and insulin (mol wt 6000). The void volume and exclusion volumes were determined respectively by dextran blue and phenol red markers.
*Significantly greater than control, $P < 0.005$.

ums containing trypsinized C5 or trypsinized leukotactic fractions from zymosan-activated rat serum were incubated with antiserums to C5. This treatment inhibited completely the chemotactic activity generated by trypsinization of purified C5 or leukotactic fractions of zymosan-activated rat serum. However, there was no discernible effect on the chemotactic activity present in medium from resorbed bones.

Cellular Specificity of the Bone-Derived Chemotactic Factor

Text-figure 3 illustrates typical chemotactic responses of two different tumor types to various dilutions of resorbed bone mediums. Maximal responses of the EL-4 cells occurred with dilute mediums; lesser responses

Table 3—Effects of Anti-C5 Antiserums on the Tumor Cell Chemotactic Activities Derived From Resorbing Bones and From C5

Chemotaxis sample	Chemotactic activity (cells/HPF ± SE)			
	Untreated sample	Trypsinized sample*	Incubated with anti-C5†	Incubated with with normal goat serum‡
Resorbed bone medium	51 ± 2¶	—	56 ± 3¶	51 ± 2¶
Purified human C5§	26 ± 2	62 ± 3¶	30 ± 3	53 ± 3¶
Control medium	32 ± 2	—	27 ± 2	27 ± 1
Leukotactic fractions from rat serum ‖	23 ± 2	43 ± 3¶	21 ± 2	36 ± 2¶
Control medium	21 ± 1	—	20 ± 1	21 ± 2

* Trypsinized by 1% (w/w) for 60 minutes at 37 C. Digestion stopped by addition of 2% soybean inhibitor.

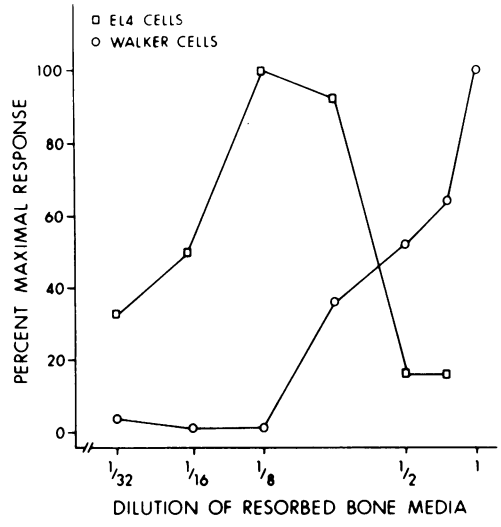
† Twenty-five microliters of antibody-containing goat serum added to 1 ml of sample and incubated at 37 C before testing for chemotactic activity.

‡ Twenty-five microliters of normal goat serum employed as in†

§ Twenty-three micrograms of C5/ml chemotaxis sample.

‖ Partially purified leukotactic fractions of zymosan-activated rat serum (50 μl/ml chemotaxis sample).

¶ Significantly greater than corresponding control, $P < 0.05$.



TEXT-FIGURE 3—Effect on migration on Walker carcinosarcoma cells and EL-4 lymphoma cells of varying the concentration of bone-resorbed medium in the lower compartment of the Boyden chamber. The dilutions were made in control mediums. The values for random migration of the cells (when control medium was placed in the lower compartment) were: Walker carcinoma cells, 18 ± 1 cells/high-power field; EL-4 lymphoma cells 5 ± 1 cells/high-power field. Maximal values observed in this experiment were: Walker carcinoma cells, 43 ± 2 cells/high-power field; EL-4 cells, 17 ± 2 cells/high-power field.

were observed when the concentration of resorbed bone medium was increased beyond that which elicited maximal responses by the cells. Loss of response of the Walker cells at lower dilutions was not observed in any of our experiments. On the basis of several such experiments it appears that the concentration of chemotactic factor required to elicit a half-maximal chemotactic response from the EL-4 cells is several times less than that required to elicit a half-maximal chemotactic response from the Walker tumor cells. Cells from a murine plasmacytoma which grows as an ascites tumor and does not metastasize to bone did not respond to the bone-derived chemotactic factor (data not shown).

Discussion

A correlation between the extent of bone resorption and the intensity of the chemotactic activity released into the culture medium suggests that the chemotactic factor is a by-product of the process of bone resorption. The concept is consistent with the observation that the generation of the bone-derived chemotactic activity for tumor cells can be induced by several agents which stimulate osteoclastic bone resorption.[10] It is possible that the chemotactic factor generated by resorbing bones could be a cell-derived humoral factor (for example, released by activated osteoclasts) or a matrix component that is released during the process of bone resorption. Either of these two possibilities is consistent with our data. All of the humoral mediators of bone resorption cause similar morphologic changes in osteoclasts which are the primary bone-resorbing cells.[18] It is conceivable

that the activated osteoclast, independent of the mediator of its activation, could synthesize and release directly a soluble chemotactic signal for tumor cells. Alternatively, the osteoclast resorbs bone in a similar manner no matter how it is stimulated. The degradation of the bone matrix due to release of hydrolytic enzymes by osteoclasts could release a chemotactic signal buried in the bone matrix. The bone matrix is largely composed of Type I collagen, which is enzymatically degraded during the process of bone resorption. Enzymatic digestion of other collagen types generates chemotactic factors for fibroblasts,[19] and in experiments not reported here we have been able to generate chemotactic activity for tumor cells by collagenase treatment of bones.

It is unlikely that calcium ions released from bone during the process of resorption are responsible for the chemotactic responses we observed. The dialysis data and gel filtration chromatography experiments suggest that chemotactic factor has a molecular weight of approximately 6000 daltons. In addition, in earlier experiments we found we could not alter tumor cell chemotaxis by changing the calcium ion concentration in the range observed in medium harvested from our resorbed bones.

In spite of a striking similarity in molecular weights,[7,9] it is also unlikely that the bone-derived chemotactic factor is the same factor as that derived from C5, since treatment of medium by serum containing antibodies to C5 blocked the chemotactic activity in medium containing trypsinized C5 but not bone-derived factors. Further evidence for the difference between these factors is that a chemotactic factor generated in resorbing bone cultures is effective against monocytes, whereas the C5-derived chemotactic factor for tumor cells is not.[20] Thus, chemotactic responses of cultured cells show specificity both in terms of the factors to which individual cell cultures will respond and the cells on which individual chemotactic factors act. No single chemotactic factor or cell type behaves exactly like any other factor or cell (Table 4).

It may prove very difficult to purify the chemotactic factor derived from bone using standard purification techniques such as molecular sieve chromatography, ion exchange chromatography and gel electrophoresis. The bones are very minute, and the amount of biologically active material released into the conditioned medium is small. In addition, the chemotactic effect may be due to a group of factors rather than one single factor released by enzymatic degradation of the bone matrix. Attempts at purification under these circumstances would be almost impossible. Furthermore, the need for a bioassay measurement of activity during purification, even one that is relatively quick and reproducible, such as the Boyden chamber technique, is likely to mean slow progress, particularly with the limitations on the amount of starting material which is available.

Table 4—Summary of the Chemotactic Responses of Serveral Normal Cell Types and Tumor Lines to Various Chemotactic Stimuli

Chemotactic factor	Neutrophils	Monocytes	Walker carcinosarcoma	EL-4 Lymphoma
Bacterial factor*	+	+	−	−
C5-derived leukotactic factor†	+	+	−	−
C5-derived factor for tumor cells‡	−	−	+	−
Bone-derived factor for tumor cells	−	+	+	+

* Media from a 24-hour culture of *E coli*.

† Chemotactic activity present in partially purified zymosan-activated serum or produced by partial trypsinization of C5.[7]

‡ Chemotactic activity produced by trypsinization of partially purified zymosan-activated serum or by trypsinization of C5 for periods longer than that required to produce leukotactic activity.[7]

It is not clear which mediator of bone resorption produced by Walker cells is responsible for bone resorption. In Walker 256 cells examined by Powles et al [11] it seemed likely that prostaglandin E was responsible. Hypercalcemia in the rats carrying the tumors was inhibited by indomethacin and aspirin, drugs that inhibit prostaglandin synthesis, and production of bone resorbing activity by cultured tumor explants *in vitro* was inhibited by incubation with these drugs. In contrast, there is evidence that Walker carcinosarcoma cells examined by Minne et al.[12] produce immunoreactive parathyroid hormone-like material. We have not defined the nature of the bone resorbing factor produced by our cultured Walker cells as yet. However, we have previously shown that both parathyroid hormone and prostaglandin E stimulated bones to release chemotactic activity for our Walker cells.[10]

The Walker carcinosarcoma is a tumor that has a marked propensity to induce osteolysis and to metastasize to bone. We propose that the selection of bone as a metastatic site may depend upon the ability of a tumor to stimulate bone resorption, thereby generating chemotactic factors from bone. Enhancement of bone resorption would not necessarily depend upon the presence of tumor cells in bone, since a number of tumors are capable of secreting humoral mediators that could induce osteoclastic bone resorption at distant sites.[21] The chemotactic responses of circulating tumor cells might then result in the migration of these cells to bone where they could develop into secondary tumor deposits.

References

1. Nicolson G: Cell and tissue interactions leading to malignant tumor spread (metastasis). Am Zool 1978, 18:71–80
2. Sugarbaker EV, Ketcham AS: Mechanisms and prevention of cancer dissemination: An overview. Semin Oncol 1977, 4:19–32

3. Sträuli P, Weiss L: Cell locomotion and tumor penetration. Eur J Cancer 1977, 13:1–12
4. Yoshida K, Ozaki T, Ushijima K, Hayashi H: Studies on the mechanisms of invasion in cancer: I. Isolation and purification of a factor chemotactic for cancer cells. Int J Cancer 1970, 6:123–132
5. Romualdez AG Jr, Ward PA: A unique complement derived chemotactic factor for tumor cells. Proc Nat Acad Sci USA 1975, 72:4128–4132
6. Romualdez AG Jr, Ward PA, Torikata T: Relationship between the C5 peptides chemotactic for leukocytes and tumor cells. J Immunol, 1976, 117:1762–1766
7. Orr W, Varani J, Ward PA: Characteristics of the chemotactic response of neoplastic cells to a factor derived from the fifth component of complement. Am J Pathol, 1978, 93:405–422
8. Orr FW, Varani J, Kreutzer DL, Senior RM, Ward PA: Digestion of the fifth component of complement by leukocyte enzymes: Sequential generation of chemotactic activities for leukocytes and for tumor cells. Am J Pathol 1979, 94:75–84
9. Orr W, Phan SH, Varani J, Ward PA, Kreutzer DL, Webster RO, Henson PM: Chemotactic factor for tumor cells derived from the C5a fragment of complement component C5. Proc Nat Acad Sci USA 1979, 76:1986–1989
10. Orr W, Varani J, Gondek MD, Ward PA, Mundy GR: Chemotactic responses of tumor cells to products of resorbing bone. Science 1979, 203:176–179
11. Powles TJ, Clark SA, Easty DM, Easty GC, Neville AM: The inhibition by aspirin and indomethacin of osteolytic tumor deposits and hypercalcemia in rats with Walker tumour, and its possible application to human breast cancer. Br J Cancer 1973, 28:316–321
12. Minne H, Raue F, Bellwinkel S, Ziegler R: The hypercalcaemic syndrome in rats bearing the Walker carcinosarcoma 256. Acta Endocrinol (Copenh) 1975, 78:613–624
13. Ozaki T, Yoshida K, Ushijima K, Hayashi H: Studies on the mechanisms of invasion in cancer: II. *In vivo* effects of a factor chemotactic for cancer cells. Int J Cancer 1971, 7:93–100
14. Raisz LG: Bone resorption in tissue culture: Factors influencing the response to parathyroid hormone. J Clin Invest, 1965, 44:103–116
15. Trummel CL, Mundy GR, Raisz LG: Release of osteoclast activating factor by normal human peripheral blood leukocytes. J Lab Clin Med 1975, 85:1001–1007
16. Nilsson UR, Tomar RH, Taylor FB Jr: Additional studies on human C5: Development of a modified purification method and characterization of the purified product by polyacrylamide gel electrophoresis. Immunochemistry 1972, 9:709–723
17. Ward PA Zvaifler NJ: Complement-derived leukotactic factors in inflammatory synovial fluids of humans. J Clin Invest 1971, 50:606–616
18. Vaughan JM: The Physiology of Bone. London, Oxford University Press, 1975
19. Postlethwaite AE, Seyer JM, Kang AH: Chemotactic attraction of human fibroblasts to type I, II, and III collagens and collagen-derived peptides. Proc Natl Acad Sci USA 1978, 75:871–875
20. Mundy GR, Varani J, Orr W, Gondek M, Ward PA: Resorbing bone is chemotactic for monocytes. Nature, 1978, 275:132–135
21. Tashjian AH Jr: Role of prostaglandins in the production hypercalcemia by tumors. Cancer Res 1978 38:4138–4141