# *Comparison of the Chemotactic Responsiveness of Two Fibrosarcoma Subpopulations of Differing Malignancy*

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**There are several points of similarity between the processes of cancer metastasis and inflammation. In both, cells circulate in the vasculature, arrest, and cross vessel walls, thereby entering the extravascular tissues. *In vitro*, leukocytes and some, but not all, tumor cells exhibit chemotaxis. Since the chemotactic responses of leukocytes effect their transvascular migration, we propose that chemotactic responsiveness contributes to the ability of circulating tumor cells to localize in extravascular tissues. This study was done to seek a relationship between chemotactic responsiveness of tumor cells and their behavior *in vivo*. Two subpopulations of cells were isolated from a methylcholanthrene-induced fibrosarcoma. The two cell lines were compared with regard to their biologic behavior *in vivo* and their chemotactic responsiveness *in vitro*. *In vivo* one subpopulation was highly malignant. An injection of $2.0 \times 10^5$ cells into the footpad of syngeneic mice led to the development of primary tumors in 87% of the animals and lung metastases in 61% of the animals with primary tumors. This line demonstrated chemotaxis to a factor that behaved similarly in gel filtration and showed immunologic reactivity similar to that of a previously described tumor cell chemotactic factor derived from the fifth component of complement. In contrast, an injection of the same number of cells from the second subpopulation of fibrosarcoma cells led to the development of primary tumors in only 12% of syngeneic mice, and lung metastases did not occur. Neither this subpopulation nor normal embryonic fibroblasts demonstrated chemotactic responsiveness. We postulate that the ability of tumor cells to respond to specific chemotactic stimuli may be one of the many unique properties which distinguish malignant from benign tumor cells. This is the first report documenting the chemotactic responsiveness of non-ascites tumors and fibrosarcomas. (Am J Pathol 1981, 102:160–167)**

THE MOST DISTINCTIVE characteristic of malignant tumors is their ability to metastisize. It is currently held that the ability of tumors to invade and metastasize can be related to potentially identifiable properties unique to malignant tumor cells and absent from benign tumor cells. Although no one of these properties alone is likely to be responsible for the behavior of the malignant tumor *in vivo*, together they endow the malignant tumor cell with the ability to complete the complex series of events in the process of metastasis.[1,2] The steps in this "metastatic cascade" include the separation of cells from the primary tumor, entry of the tumor cells into the blood or lymphatic circulatory pathways, exit of surviving cells from the circulation, and proliferation of these cells in the extravascular tissues.[3-5] There are several points of resemblance between this sequence and that which leads to the accumulation of leukocytes at foci of inflammation. For example, leukocytes and tumor cells enter the circulation from an extravascular site, stop at distant points within the circulation, and leave the circulation to enter the extravascular tissues. Since the chemotactic responses of leukocytes effect the directed migration of these cells into sites of inflammation,[6] the similarities between metastasis and the inflammatory process suggest the hypothesis that analogous mechanisms may bring about the transvascular migration of tumor cells, accounting for the localization of secondary tumors.

*In vitro*, cells from a number of different ascites tumors have been shown to exhibit the property of chemotactic responsiveness to factors derived from tumor tissue,[7] the fifth component of serum comple-

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ment,[8] and resorbing bone.[9] We have shown recently that a complement-derived chemotactic factor for tumor cells can be generated by proteolytic digestion of the fifth component of complement, the C5a-containing leukotactic fraction of activated serum,[10] or highly purified anaphylatoxin C5a.[11] This chemotactic factor has a molecular weight of approximately 6000 daltons.[10,11] Its activity is blocked by incubation with antiserum to intact C5.[12] A number of different transmissible ascites tumor cells exhibit chemotactic responsiveness to the C5-derived factor.[13] However, the lack of a demonstrable chemotactic response to this factor by a murine lymphoma cell line[10] indicated that not all tumor cells exhibit responses to the C5-derived chemotactic factor for tumor cells. Furthermore, with the exception of monocytes, which respond to chemotactic factors derived from resorbing bone,[14] leukocytic cells do not respond to those preparations of factors that are chemotactic for tumor cells.[7,9,10] These observations indicate that both leukocytes and tumor cells exhibit considerable selectivity in their responses to various chemoattractants.

One problem posed by these observations is to relate the chemotactic responsiveness of tumors *in vitro* to their biological behavior *in vivo*. Thus we wish to determine whether chemotactic responsiveness is a characteristic that can be correlated with malignancy. In this study our approach to this problem was to examine the relationship between chemotactic responsiveness and the behavior *in vivo* of three closely related cell types of fibroblastic origin. We report here that a highly malignant murine fibrosarcoma is chemotactically responsive to a factor having the same properties as the previously described C5-derived chemotactic factor for tumor cells. A closely related, nonmalignant fibrosarcoma (derived from the same parent tumor) and normal, syngeneic fibroblasts do not respond to this factor. This is the first report demonstrating the chemotactic responsiveness of non-ascites tumors and fibrosarcomas.

## Materials and Methods

### Fibrosarcoma Cells and Mouse Embryonic Cells

The fibrosarcoma cell lines were established from a tumor induced in a C57 bl/6 mouse by 3-methylcholanthrene. A parent cell line, derived directly from this tumor, was cultured *in vitro* for approximately 2 years prior to the establishment of the 2 subpopulations employed in this study.[15] The parent cells were maintained in Medium 199 supplemented with 10% fetal calf serum, 50 U/ml penicillin, and 50 μg/ml streptomycin. Cells were maintained at 37 C in a humidified atmosphere containing 5% $CO_2$.

*Fibrosarcoma Subpopulation A*

Syngeneic mice 4–8 weeks old, (C57 bl/6 females) were injected in the right rear footpad with $1.5 \times 10^5$ viable tumor cells from the parent fibrosarcoma line. After 35 days the animals were killed by ether anesthesia, the lungs removed aseptically and examined for metastases. One of the metastatic tumor nodules was placed into monolayer tissue culture. This subpopulation has been maintained in culture for over 1 year.

*Fibrosarcoma Subpopulation B*

In a previous report we described the migratory characteristics of the parent fibrosarcoma cells when suspended in medium with either fetal calf serum or normal human serum.[15] The cells migrated less actively in human serum than in fetal calf serum. We subsequently found that when the cells were suspended as single cells in medium with either human serum or fetal calf serum, the number of cells that attached to plastic flasks was much lower in the presence of human serum.[16] However, by continually selecting those cells that did attach in this medium, we were able, over the course of 4–5 months, to establish a population of cells that would routinely attach to the flasks in medium with normal human serum and would grow to monolayers. We have recently described the migrational characteristics, attachment capabilities, and activity of protease and glycosidase enzymes in these cells.[17] These cells have been maintained for over 1 year in medium with 10% fetal calf serum but have retained the *in vitro* characteristics noted above.

*Mouse Embryonic Cells (Fibroblastlike Cells)*

Mouse embryonic cells were prepared from 14-day-old C57 bl/6 embryos by a method modified from that of Duff and Rapp.[18] Embryos from a single litter were pooled, minced, and dispersed with 0.5% 1:250 trypsin (Difco Laboratories, Detroit, Michigan) for a period of 30 minutes at room temperature. Afterwards, the tissues were washed 3 times in Medium 199. Flasks 75 sq. cm were seeded with $10^7$ cells suspended in Medium 199 with 10% fetal calf serum. These were incubated at 37 C in a humidified atmosphere with 5% $CO_2$. When the surface of the flask was covered by a confluent monolayer of cells, the flasks were trypsinized, and sub-cultures were established at a split ratio of 1:2. Cells from these subcultures were employed in experimental studies.

For chemotaxis studies, cultured cells were removed from the flasks mechanically, rather than by trypsinization,[10] centrifuged at 250*g* for 10 minutes to remove them from the original tissue culture medium, and resuspended in medium composed of an equal volume of the original (conditioned) medium and fresh Medium 199 with 10% fetal calf serum. The final cell concentration was $1 \times 10^6$ cells/ml.

### Walker Ascites Tumor Cells

The Walker carcinosarcoma was maintained as an ascites tumor by serial passage. The preparation of these cells for chemotaxis studies was described in detail previously.[10]

### Human Peripheral Blood Leukocytes

Human peripheral blood leukocytes were obtained by dextran sedimentation of heparinized whole blood. The granulocytes were isolated on a Ficoll-Hypaque gradient after erythrocytes had been removed by osmotic lysis of the dextran-sedimented cells. For studies of chemotaxis, granulocytes were suspended at a concentration of $2.5 \times 10^6$ cells/ml in Medium 199, containing 2 mg/ml bovine serum albumin. The technique for studying leukocyte chemotaxis has been described.[8]

### *In Vivo* Behavior of Fibrosarcoma Cells

Syngeneic mice (4–8 weeks old, C57 bl/6 females) were injected in the right rear footpad with $2 \times 10^5$ viable tumor cells from each subpopulation. Animals were observed at intervals of 4–5 days for signs of tumor growth at the site of injection. The size of the injected foot was also measured with calipers. After 35–40 days, the animals were killed by ether anesthesia, and the lungs were examined for metastatic nodules after intratracheal injection of Bouin's solution.[19]

### Chemotactic Factors

The fraction containing chemotactic activity for leukocytes (partially purified C5a) was obtained from zymosan-activated serum by a previously described modification of the method of Vallota et al.[10,20] In order to generate chemotactic activity for tumor cells, this material was treated with 1% trypsin (w/w) based upon the total protein concentration in the sample, determined by the method of Lowry et al.[21] Trypsinization was conducted for a period of 30 minutes at 37 C in an agitating water bath. Soybean trypsin inhibitor (2% w/w) was added to each sample to inhibit the action of trypsin at the end of the digestion. The supernatant fluids from cultures of *Escherichia coli* were prepared as described by Ward et al.[22]

### Assays of Cell Migration and Chemotaxis

The method of Romualdez and Ward[8] was modified as follows: the micropore filters used for studies of tumor cell migration were composed of nitrocellulose with a porosity of 12 $\mu$ (Selection Filters, Schleicher and Schuell, Keene, NH). Each assay of tumor cell chemotaxis was performed in triplicate and the number of cells migrating in three high-power fields was determined on each filter. The mean and standard error were calculated from the values thus obtained. Statistical analysis was performed by means of the Student *t* test.

### Gel Filtration

Sephadex G75 (Pharmacia Fine Chemicals, Piscataway, NJ) was prepared as directed by the manufacturer and poured into a column measuring 60 cm by 1.6 cm with a bed volume of 120 ml. The column was eluted with Hanks' balanced salt solution, pH 7.4, and calibrated with blue dextran (mol wt $2 \times 10^6$), cytochrome C (mol wt $1.3 \times 10^4$), and phenol red (mol wt $3.5 \times 10^2$). Five milliliters of the trypsinized or nontrypsinized leukotactic fraction of activated serum was applied to the column. Fractions of 5 ml were collected. For chemotaxis assay, 100 $\mu$l of each fraction was diluted with Medium 199 to a volume of 1 ml and placed into the lower compartment of a Boyden chamber.

### Antibody Blocking Experiments

Antibodies to C5 were raised in goats and were characterized as previously described.[23] Antiserums to the third component of human complement (C3) and to whole human serum were purchased from Behringwerke AG, (Marburg, West Germany). In experiments designed to test the suppressive effects of the antiserums on preformed chemotactic activity, 25-$\mu$l volumes of antiserum or normal goat serum were incubated at 37 C for 30 minutes with 75 $\mu$l of the trypsin-treated leukotactic fraction of activated serum and then brought to a volume of 1 ml with Medium 199. The antibody-treated chemotactic factors were then assayed in Boyden chambers. Additional

controls consisted of antiserums in Medium 199 without the presence of the trypsin-treated leukotactic fraction of activated serum.

## Results

### *In Vivo* Behavior of Fibrosarcoma Cells

The results of 5 separate experiments are summarized in Table 1. In the groups of animals injected with cells from fibrosarcoma subpopulation A, tumors occurred at the site of footpad injection in 33 of 38 animals. Most of these tumors were visible by the tenth day after injection. At the time of terminating the experiment, the footpad tumors produced by injection of fibrosarcoma subpopulation A were 4–16 mm in diameter. Twenty of the 33 animals with tumors at the site of injection had pulmonary metastases. The average number of pulmonary tumor nodules in these animals was 8 ± 4. Lung tumors were not found in any animal that did not have a footpad tumor.

Tumors occurred in the footpad of 6 of the 50 animals given injections of cells from fibrosarcoma subpopulation B. These tumors were not visible until 20–30 days after injection. After 35–40 days the footpad tumors produced by injecting cells from fibrosarcoma subpopulation B were only 1–2 mm in diameter. None of the animals given cells from this subpopulation had grossly visible lung metastases, even when primary tumors were allowed to grow until they were the same size as the tumors produced by subpopulation A.

### Chemotactic Responses of the Cell Lines

The migration of the cell lines was examined under conditions in which variations were made in the concentration of the different chemotactic stimuli in the lower compartment of the Boyden chambers. The nontrypsinized leukotactic fraction of activated serum and the trypsinized leukotactic fraction of activated serum were employed as chemoattractants for leukocytes and for tumor cells, respectively. The effect of different doses of these two factors on the migration of human peripheral leukocytes and Walker ascites tumor cells is indicated in Figure 1. While the leukotactic fraction of activated serum (partially purified C5a) was a highly potent chemoattractant for leukocytes, it effected no demonstrable change in the numbers of the Walker ascites tumor cells migrating into the micropore filter. Conversely, the trypsinized leukotactic fraction of activated serum was chemotactic for the Walker ascites tumor cells but not for leukocytes.

A marked chemotactic response of the fibrosar-

Table 1—Comparison of the *in Vivo* Behavior of Cells From the Two Fibrosarcoma Subpopulations

| Cell Line | n* | Incidence of primary tumors | Incidence of pulmonary metastases† |
|---|---|---|---|
| Fibrosarcoma subpopulation A | 38 | 85 ± 9% | 61 ± 11%‡ |
| Fibrosarcoma subpopulation B | 50 | 8 ± 5%§ | 0%§ |

* Total number of animals employed in 5 separate experiments.
† Numbers refer to the percentage of primary tumor-bearing animals that had pulmonary metastases.
‡ Average number of pulmonary nodules was 8 ± 4 per animal.
§ Significantly less than incidence associated with fibrosarcoma subpopulation A ($P < 0.001$).

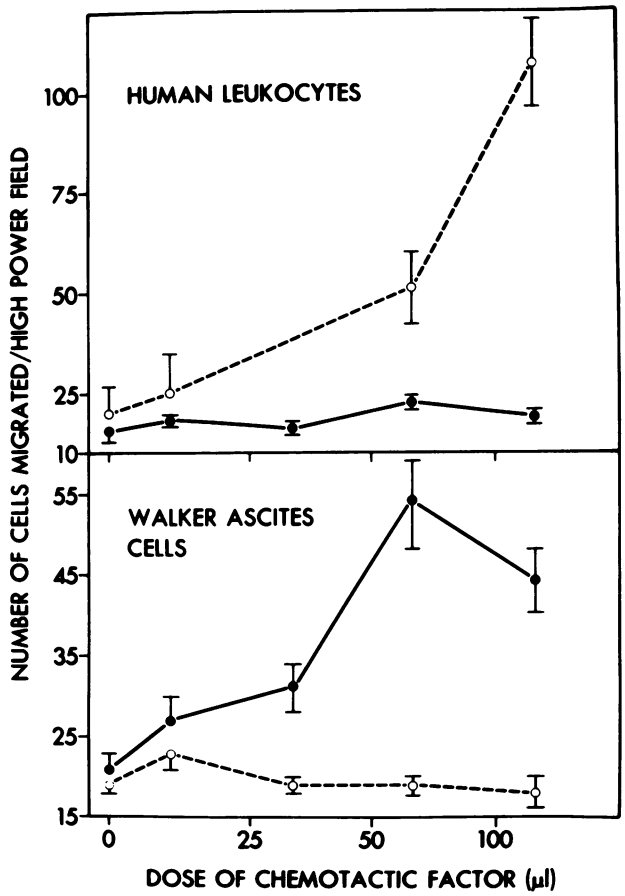


**Figure 1**—Effect on migration of human leukocytes and Walker ascites tumor cells of various doses of chemotactic factors. The dose of chemotactic factor represents the volume of chemotactic factor in 1 ml of culture medium placed into the lower compartment of the Boyden chamber. Two chemotactic factors were used in these experiments: the leukotactic fraction of activated serum (O - - - O) and the trypsin-treated leukotactic fraction of activated serum (●——●). The values expressed for cell migration are the mean ± standard error from a representative experiment.

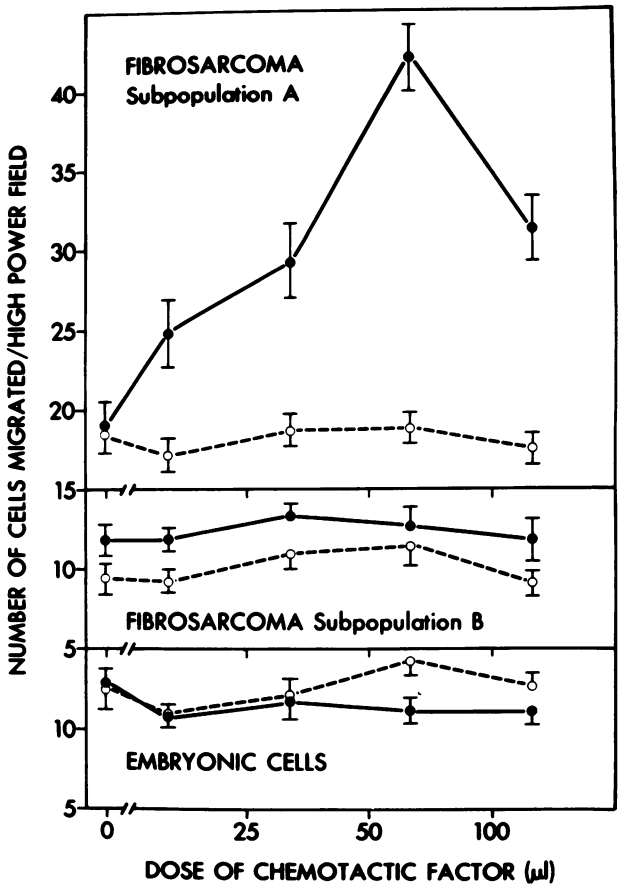


**Figure 2**—Effect on migration of fibrosarcoma and embryonic cell lines of various doses of chemotactic factor. The dose of chemotactic factor represents the volume of chemotactic factor in 1 ml of culture medium placed into the lower compartment of the Boyden chamber. Two chemotactic factors were used in these experiments: the leukotactic fraction of activated serum (O - - - O) and the trypsin-treated leukotactic fraction of activated serum ( ●——● ). The values expressed for cell migration are the mean ± standard error of two separate experiments.

coma subpopulation A was observed when the trypsinized leukotactic fraction of activated serum was employed as a chemoattractant (Figure 2). The dose of chemotactic factor that elicited this response was similar to that which induced a response of the Walker ascites tumor cells. For both cell types a migratory response less than maximal was produced by doses of chemotactic factor greater than those which elicited a maximal migratory response. Cells from fibrosarcoma subpopulation B and the embryonic cells did not demonstrate chemotactic responsiveness to either the nontrypsinized or the trypsinized leukotactic fraction of activated serum.

Human leukocytes demonstrated a chemotactic response to the supernatant fluids from cultures of *E coli*. The dose of this factor required to produce a 50% maximal response was 10 μl. When this factor was tested over a dose range from 1 to 1000 μl per milliliter of chemotactic fluid, no significant alteration was observed in the migration of fibrosarcoma cells, embryonic cells, or Walker ascites tumor cells (data not shown).

### Properties of the Chemotactic Factor for Fibrosarcoma Cells

These experiments were conducted in order to determine whether the chemotactic factor for fibrosarcoma subpopulation A had a different behavior in gel filtration or different immunologic reactivity from the C5-derived chemotactic factor for Walker ascites tumor cells.

Following gel filtration of the trypsinized leukotactic fraction of activated serum on Sephadex G75, the chemotactic activities for fibrosarcoma subpopulation A and for Walker ascites tumor cells were located in the same position on the chromatogram (Figure 3). The fractions containing these activities were beyond the cytochrome C marker, close to the exclusion limits of the column. We have previously shown that these fractions correspond to a molecular weight of approximately 6000 daltons.[10,11] As indicated in Figure 3, the location of the tumor cell chemotactic activity was distinctly different from the location of the leukotactic activity present in the untrypsinized leukotactic fraction of activated serum. The latter activity is attributed to C5a, which has a molecular weight of approximately 11,000 daltons.[24]

Experiments were done in order to determine whether blocking of the chemotactic activity for fibrosarcoma subpopulation A could be achieved by incubation of the preformed chemotactic factors with antiserums to various components of normal serum (Table 2). Incubation of the trypsinized leukotactic fraction of activated serum with antiserums to C5, or with polyvalent antiserums to whole human serum, inhibited completely the chemotactic activity for fibrosarcoma subpopulation A and for Walker ascites tumor cells. Incubation of the trypsin-treated leukotactic fraction of activated serum with antiserum to C3 or with normal goat serum had no effect on the preformed chemotactic activity for the tumor cells.

## Discussion

The exit of circulating neoplastic cells from the blood stream or lymphatic vessels is a major step in the establishment of secondary tumors. Observations on this phase of tumor metastasis have indicated that it may be effected by several different mechanisms. It is possible that some tumors may penetrate and de-

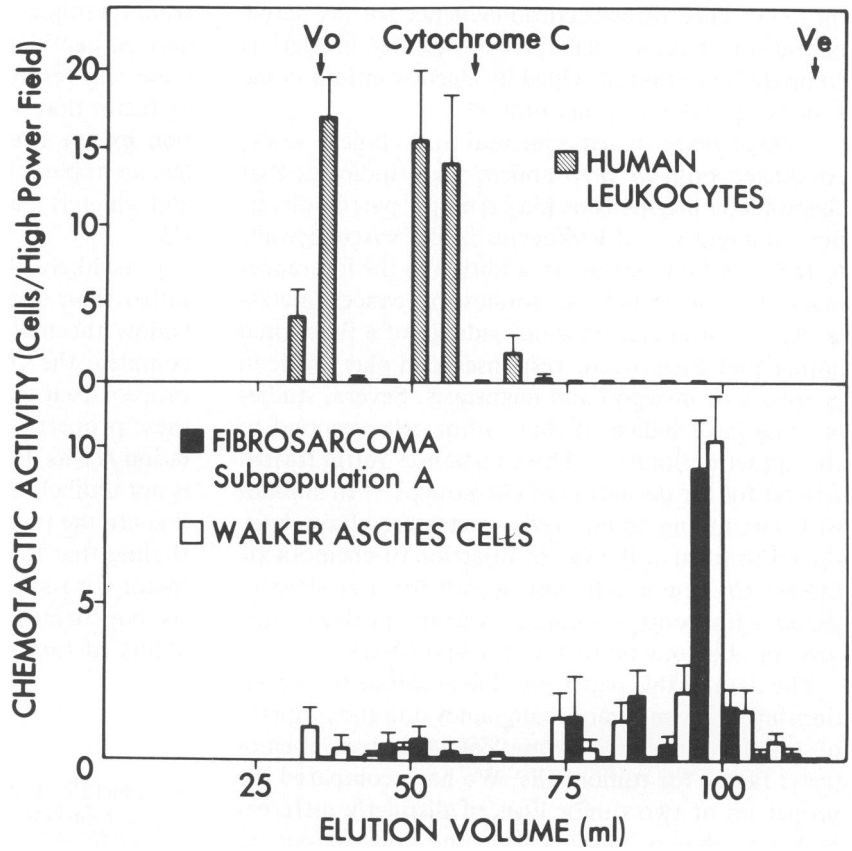


**Figure 3**—Behavior in gel filtration on Sephadex G75 of the chemotactic activities for leukocytes and for tumor cells. In the experiment depicted in the upper frame, the leukotactic fraction of activated serum was chromatographed and each fraction tested for chemotactic activity for human peripheral leukocytes. In the experiments depicted in the lower frame, the leukotactic fraction of activated serum was trypsinized and then chromatographed on the same column as that employed for the experiment in the upper frame. The fractions were then tested for chemotactic activity for fibrosarcoma subpopulation A and the Walker ascites tumor cells. The values expressed in this figure represent the mean of the total number of cells migrated per high-power field when fluid from each fraction was tested for chemotactic activity minus the mean number of cells migrated per high-power field when culture medium alone was tested for chemotactic activity (random migration). The values for random migration were: leukocytes 16 ± 3 cells per high-power field; fibrosarcoma subpopulation A 21 ± 5 cells per high-power field; Walker ascites tumor cells 23 ± 2 cells per high-power field.

stroy the vascular endothelium and basement membrane. By so doing, they could gain direct access to the extravascular tissues.[25] Alternatively, at least some tumor cells are capable of active migration across the vessel wall. Evidence for this second mechanism includes direct observations made by the skin window technique, showing the penetration of endothelium by emigrating tumor cells. These studies *in vivo* suggest that mechanisms of endothelial destruction and active migration may operate together.[26] Electron microscopic studies of developing metastases have demonstrated extension of the pseudopods of intravascular tumor cells through the intercellular junctions between the endothelial

Table 2—Effect of Various Antiserums on the Chemotactic Activity for Tumor Cells Generated by Trypsinization of the Leukotactic Fraction of Activated Serum

| | Treatment of chemotactic factor* | | | | |
|---|---|---|---|---|---|
| Chemotactic factor | None | Anti-C5 | Anti-C3 | Anti-whole serum | Normal goat serum |
| Fibrosarcoma subpopulation A cells migrated/HPF† | | | | | |
| Trypsinized leukotactic fraction of activated serum‡ | 52 ± 2§ | 21 ± 1¶ | 48 ± 2§ | 23 ± 1¶ | 42 ± 2§ |
| Medium 199 | 25 ± 1 | 22 ± 1 | 23 ± 1 | 23 ± 1 | 27 ± 1 |
| Walker ascites tumor cells migrated/HPF† | | | | | |
| Trypsinized leukotactic fraction of activated serum | 31 ± 2§ | 12 ± 1¶ | 29 ± 2§ | 16 ± 1¶ | 32 ± 2§ |
| Medium 199 | 15 ± 1 | 13 ± 1 | 17 ± 1 | 12 ± 1 | 14 ± 1 |

* 25 μl of each antiserum or normal goat serum was incubated with 1 ml of chemotactic factor for 30 minutes at 37 C prior to the performance of the chemotaxis assay.

† Results are the mean ± SE of two separate experiments.

‡ 75 μl of trypsin-treated leukotactic fraction of activated serum per milliliter chemotactic fluid.

§ Significantly greater than corresponding control ($P < 0.001$).

¶ Not significantly different from corresponding control ($P > 0.05$).

cells.[27,28] This ultrastructural evidence for the active migration of tumor cells across vascular barriers is comparable to that provided by electron-microscopic studies of leukocyte migration.[29]

A large body of experimental and clinical work, conducted both *in vitro* and *in vivo*, indicates that chemotactic mechanisms play a major part in directing the migration of leukocytes across vascular walls to foci of inflammation. In addition to the inferences made by the structural studies of cancer metastases,[26-28] there is increasing evidence of a functional nature that chemotactic responses also play a role in processes of invasion and metastasis. Several studies *in vitro* have indicated that tumor cells respond to chemotactic stimuli.[7-9] These responses fulfill formal criteria for the definition of chemotaxis.[10] In animals with circulating tumor cells, metastases have been shown to form at the site of injection of chemotactic factors for tumor cells but do not form at sites injected with control medium, vasopermeability factors, or chemotactic factors for leukocytes.[30]

The data in this paper provide evidence for a relationship between tumor malignancy and the property of responsiveness to a complement-derived chemotactic factor for tumor cells. We have compared the properties of two tumor lines of distinctly different biologic behavior derived from the same parent tumor. Chemotactic responsiveness to a factor derived from C5 was observed only in the cell line characterized by an aggressive behavior (rapid local growth) and the propensity to metastasize. Since in our experiments, normal murine embryonic cells did not respond to partially purified C5a or to the C5-derived chemotactic factor for tumor cells, one may speculate that the ability to respond to the chemotactic factor for tumor cells is a property unique to neoplastic cells. However, since neither the normal, murine embryonic cells (fibroblastlike cells) nor the tumor subpopulation of low malignancy responded to the chemotactic stimulus, our data could indicate that responsiveness of tumor cells to chemotactic factors is a property associated with invasiveness and the ability to metastasize rather than a property associated with the neoplastic state as such. It is possible that the embryonic cells may not be a strictly comparable population of normal cells. Therefore, an additional approach to the question concerning the relationship between neoplastic transformation and responsiveness to chemotactic factors for tumor cells will be to compare the properties of cells transformed *in vitro* with nontransformed cells derived from the same population.

Human dermal fibroblasts have been shown to be capable of responding to chemotactic stimuli derived from lymphocytes[31] or from collagens and collagen-derived peptides.[32] It has been reported recently that these cells respond also to a serum-derived chemotactic factor that is generated during complement activation by the classical or alternative pathway, which has an apparent molecular weight of 80,000 daltons and which is inactivated by nonspecific antiserums to C5.[33]

It is likely that many properties and functions, rather than one single characteristic, act together to endow the malignant cell with the requirements to complete the complex sequence of metastasis. The process itself may serve as a selection mechanism for these properties.[34] Since the fibrosarcoma subpopulation A was derived from a pulmonary metastasis, it is not unlikely that some of the characteristics of this line are the result of such *in vivo* selection. Thus the finding that this line is responsive to a chemoctactic factor for tumor cells is consistent with the hypothesis that chemotactic responsiveness contributes to the ability of tumor cells to metastasize.

## References

1. Hart IR: The selection and characterization of an invasive variant of the B16 melanoma. Am J Pathol 1979, 97:587-600
2. Fidler IJ, Cifone MA: Properties of mestastatic and nonmetastatic cloned subpopulations of an ultraviolet-light-induced murine fibrosarcoma of recent origin. Am J Pathol 1979, 97:633-648
3. Sugarbaker EV, Ketcham AS: Mechanisms and prevention of cancer dissemination: An overview. Semin Oncol 1977, 4:19-32
4. Weiss L: A pathobiologic overview of metastasis. Semin Oncol 1977, 4:5-17
5. Nicholson GL: Cell and tissue interactions leading to malignant tumor spread (metastasis). Am Zool 1978, 18:71-80
6. Weiner S, Lendvai S, Rogers B, Urivetzky M, and Meilman E: Nonimmune chemotaxis *in vivo*: Inhibition by complement depletion with cobra factor. Am J Pathol 1973, 73:807-816
7. 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
8. Romualdez AG Jr, Ward PA: A unique complement derived chemotactic factor for tumor cells. Proc Natl Acad Sci USA 1975, 72:4128-4132
9. Orr FW, Varani J, Gondek MD, Ward PA, Mundy GR: Partial characterization of a bone-derived chemotactic factor for tumor cells. Am J Pathol 1980, 99: 43-52
10. 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
11. 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 Natl Acad Sci USA 1979, 76:1986-1989

12. 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
13. Romualdez AG, Ward PA: Further studies on the C5-derived chemotactic factor for tumor cells. Prog Clin Biol Res 1976, 9:65–71
14. Mundy GR, Varani J, Orr W, Gondek MD, Ward PA: Resorbing bone is chemotactic for monocytes. Nature 1978, 275:132–135
15. Varani J, Orr W, Ward PA: A comparison of the migration patterns of normal and malignant cells in two assay systems. Am J Pathol 1978, 90:159–172
16. Varani J, Orr W, Ward PA: Comparison of cell attachment and caseinolytic activities of five tumor cell types. J Cell Sci 1978, 34:133–144
17. Varani J, Orr W, Ward PA: Comparison of subpopulations of tumor cells with altered migratory activity, attachment characteristics, enzyme levels and *in vivo* behavior. Europ J Cancer 1979, 15:585–592
18. Duff R, Rapp F: Quantitative characteristics of the transformation of hamster cells by PARA (Defective simian virus 40)—Adenovirus 7. J Virol 1970, 5: 568–577
19. Fidler IJ: General considerations for studies of experimental cancer metastasis, Methods in Cancer Research. Edited by H Busch, Vol XV. New York, Academic Press, 1978, pp 399–439
20. Vallota EH, Müller-Eberhard HJ: Formation of C3a and C5a anaphylatoxins in whole human serum after inhibition of the anaphylatoxin inactivator. J Exp Med 1973, 137:1109–1123
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 1951, 193:265–275
22. Ward PA, Lepow IH, Newman LJ: Bacterial factors chemotactic for polymorphonuclear leukocytes. Am J Pathol 1968, 52:725–736
23. Ward PA, Zvaifler NJ: Complement-derived leukotactic factors in inflammatory synovial fluids of humans. J Clin Invest 1971, 50:606–616
24. Fernandez HN, Hugli TE: Partial characterization of human C5a anaphylatoxin. I. Chemical description of the carbohydrate and polypeptide portions of human C5a. J Immunol 1976, 117:1688–1694
25. Wallace AC, Chew EC, Jones DS: Arrest and extravasation of cancer cells in the lung, Pulmonary Metastasis. Edited by L Weiss, HA Gilbert). Boston, G. K. Hall, 1979, p 26
26. Wood S, Jr: Pathogenesis of metastasis formation observed in vivo in the rabbit ear chamber. Arch Pathol 1958, 66:550–568
27. Dingemans KP: Invasion of liver tissue by blood-borne mammary carcinoma cells. J Natl Cancer Inst 1974, 53: 1813–1824
28. Sindelar WF, Tralka TS, Ketcham AS: Electron-microscopic observations on formation of pulmonary metastases. J Surg Res 1975, 18:137–161
29. Marchesi VT: The site of leukocyte emigration during inflammation. Q J Exp Physiol 1961, 46:115–118
30. 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
31. Postlethwaite AE, Snyderman R, Kang AH: The chemotactic attraction of human fibroblasts to a lymphocyte-derived factor. J Exp Med 1976, 144:1188–1203
32. 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
33. Postlethwaite AE, Snyderman R, Kang AH: Generation of fibroblast chemotactic factor in serum by activation of complement. J Clin Invest 1979, 64:1379–1385
34. Fidler IJ: Selection of successive tumor lines for metastasis. Nature New Biol 1973, 242:148–149

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