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Chemotaxis of metastatic tumor cells

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Summary

A number of factors have been identified which are chemotactic for tumor cells. Recent studies have shown that, in addition to inducing directional motility in the Boyden chamber assay, these factors also induce a number of other responses. Included among these responses are cell swelling and foreign surface adhesiveness. The adherence response has been studied in detail using the Walker 256 carcinosarcoma cells and several other cell types. In the Walker cells, treatment with the C5a-derived tumor cell chemotactic peptide, the synthetic tripeptide, N-formyl-methionyl-leucyl-phenylalanine or with 12-O-tetradecanoyl phorbol ester induces a rapid, transient adherence response. The response is completely inhibited by several agents known to block the activity of phospholipase A_2 or the metabolism of arachidonic acid through the lipoxygenase pathway but is not inhibited by inhibition of the cychlooxygenase pathway. This suggests that lipoxygenase metabolites of arachidonic acid may actually mediate the adherence response.

It has been shown that chemotactic factor treatment of animals that are bearing circulating tumor cells induces a localization of these cells at the site of chemotactic factor injection. On the basis of these observations it has been hypothesized that tumor cells respond to chemotactic factors in much the same way that leukocytes do and that tumor cell localization at metastatic sites in vivo may be influenced by chemotactic factors in much the same way that leukocyte localization at inflammatory sites is.

Introduction

Recent studies in a number of laboratories have demonstrated that non-mylogenous cells (both normal and neoplastic) are capable of responding to chemotactic factors in vitro (1–10). How these responses affect the functioning of the cells in vivo is not known. With normal fibroblasts it has been postulated that the ability to respond to chemotactic stimuli influences their behavior in the processes of wound healing and inflammation (1–3). Work in our laboratory has been concerned with the chemotactic responses of tumor cells and the possible influences of these responses on the process of metastasis formation. It is our working hypothesis that the localization of circulating, chemotactically-responsive tumor cells at secondary sites in vivo is influenced by chemotactic factors in much the same way that leukocyte localization at inflammatory sites is.

Although stimulation of cell motility is implicit in the concept of chemotaxis, it should be pointed out that chemotactic factors also induce a number of other physiological responses in the same cells. With leukocytes, for example, chemotactic factors induce cell-to-cell aggregation (11), cell swelling

(12), hyperadherence to foreign surfaces (13), lysosomal enzyme release (14), and a respiratory burst resulting in the production of reactive oxygen species (15). These responses may all be relevant to the functioning of leukocytes in vivo. Likewise, functional responses other than motility also occur in stimulated tumor cells (16–19). Of particular interest has been the finding that chemotactic factors induce a transient foreign surface adherence response in tumor cells (18, 19). This response may play a critical role in the process by which circulating tumor cells become localized at secondary sites in vivo.

In this review I will describe briefly what is known about the functional responses, particularly the hyperadherence response, of tumor cells to chemotactic factors. Included will be a discussion of the biological basis (in so far as it is known) of these responses as well as possible regulatory mechanisms. Finally, findings that indicate an in vivo role for these responses will also be presented and attempts will be made to synthesize a coherent (though as yet speculative) hypothesis to explain how the chemotactic activation of tumor cells could influence their behavior in vivo.

Chemotactic activation of tumor cells in vitro

Chemotaxis is defined as the directional migration of cells toward an increasing concentration of an attracting chemical. The standard way to measure this response in vitro is with the Boyden chamber assay, although other techniques are also available (20, 21). In the Boyden chamber assay a micropore filter is used to separate an upper and lower fluid-filled compartment. The cells to be tested are placed on the surface of the filter in the upper compartment and the chemotactic factors is placed in the lower compartment. Cell migration into the pores of the filter is then measured.

Using this technique, a number of factors have been identified which are chemotactic for one or more types of tumor cells. Among these are factors obtained from tumor tissue (4, 5, 22), a factor found in the supernatant fluids of bone cultures that have undergone metabolic resorption (9, 23), lathyritic chick collagen, and collagen-derived peptides (10, 24), a fragment obtained by limited proteolytic digestion of the C5a leukotactic peptide (8, 25), and the synthetic trypeptide N-formyl-methionyl-leucyl-phenylalanine (FMLP) (16, 17).

The tumor tissue factors were the first chemotactic factors for tumor cells to be described. They were isolated by Hayashi and his co-workers during the early 1970s (4, 5, 22). At least two distinct factors were identified. One appeared to be a 70000 d protein while the other was a small (14000 d) molecule. It was interesting that these factors could not be obtained from isolated tumor cells but were obtainable from solid tumor tissues. Additional studies suggested that the chemotactic factors were produced from one or more proteins by the action of tumor cell proteases (26, 27). Neither of these two factors has been identified.

The factor obtained from cultures of resorbed bone has also not been positively identified. It is known to be a small molecular weight (approx. 6000 d) peptide that is antigenically unrelated to the C5a-derived peptide (9, 23). The bone-derived factor is produced in culture when rodent long bones are induced to undergo resorption with a variety of agents including parathyroid hormone, vitamin D metabolites, or prostaglandin E_1. The amount of activity produced is directly related to the degree of bone resorption but appears to be independent of the mediator of resorption. Although it is yet to be positively identified, the factors obtained during bone breakdown may be related to collagen. Recent studies have shown that native collagen, intact α collagen chains, and di and tri peptides derived from collagen are chemotactic for a variety of normal and malignant cell types. What is interesting is the fact that the spectrum of tumor cells which respond to the collagen peptides appears to be very similar to the spectrum of cells that respond to the bone factor (9, 10, 24). The non-mineral fraction of the bone matrix is, of course, nearly all collagen.

The C5a-derived tumor cell chemotactic peptide was originally isolated by Romualdez and Ward from the purified fifth component of human complement (C5) by proteolytic treatment (6). The active chemotactic factor was also obtained by

proteolytic treatment of the leukotactic fraction of zymosan-activated human serum, suggesting a relationship to the leukocyte chemotactic factor from C5 (7). Its relationship to the leukocyte chemotactic factor from C5 was further suggested by the fact that when leukocyte chemotactic factor-containing fractions of human serum or purified C5 were treated with proteolytic enzymes, leukocyte chemotactic activity decreased as the tumor cell chemotactic activity was formed (7, 8, 28). Finally it was possible to demonstrate the generation of activity by controlled proteolytic digestion of highly purified C5a (25). The tumor cell factor appears to have a molecular weight of 6000 d, making it approximately one-half the size of the native C5a molecule. Studies are currently in progress to determine which sequence of amino acids from the C5a molecule is chemotactic for the tumor cells.

In addition to these naturally occurring chemotactic factors, recent studies have shown that the potent, synthetic, leukocyte chemotactic peptide FMLP is also chemotactic for certain tumor cell types (16, 17). The fact that certain tumor cells respond to this synthetic peptide is very useful from a practical standpoint. It is possible, for example, to obtain the peptide commercially in a highly purified form. It can also be obtained as a radiolabeled derivative. Finally, a large number of analogues (with varying degrees of biological activity) as well as specific antagonists are also commercially available. Thus, this peptide may play a central role in the tumor cell model (as it already has with leukocytes) in delineating the biological basis of the cellular response to stimulation.

Because the chemotaxis assay measures the migration of cells into the tortuous pores of synthetic micropore filters, it normally takes several hours to complete. However, other types of studies show that the tumor cells respond very rapidly to stimulation with chemotactic factors. In both the cell swelling and foreign surface adherence assays, response to either the C5a-derived peptide or to FMLP can be detected within 1–3 min (16–18). This rapid response is similar to what is observed with leukocytes (12, 13). Although it has not yet been conclusively shown that the tumor cells have high-affinity receptors to either peptide (studies are in progress in our laboratory to demonstrate that they do), previous studies with leukocytes have clearly shown that the cellular responses to FMLP follow the binding of the peptide to high-affinity receptors (29). The similarities between the responses in leukocytes and the responses in the tumor cells would suggest that the responses of the tumor cells are also receptor mediated.

Stimulated adherence of tumor cells

For the past few years our laboratory has focused its attention on the stimulated adherence response of tumor cells. This response is of interest because it is probably more basic than chemotaxis per se. In addition, several recent studies have shown a direct correlation between adhesiveness and metastatic capability (30–35). Most of our studies have been conducted using the Walker 256 carcinosarcoma cells as a model. However, a number of other tumor cell types as well as normal fibroblasts have also been routinely used to determine how widespread findings made with the Walker cells are.

Two different procedures have been used to measure adherence. One procedure uses nylon fibers packed in columns as the substrate. This is similar to the method of MacGregor et al. (36). In this method, cells in suspension are treated with an appropriate factor and added to prewashed columns. After incubation for 3 min on the column, the nonadherent cells are washed out with phosphate buffered saline and counted. In this procedure, only those cells which are able to form and maintain bonds with the substrate during the 3 min of incubation are retained. In the second procedure, the cells are added to a variety of substrates, including plastic culture dishes, collagen-coated dishes and endothelial cell-coated dishes and incubated for varying lengths of time. After the incubation period, the nonadherent cells are removed and counted. This procedure differs from the first in that the cells are in contact with the substrate for a much longer period of time (up to 24 hr). Both procedures have been described in detail in our recent reports (18, 19).

For these studies the tumor cell chemotactic peptide derived from C5a and FMLP have been used. In addition to these we have used the potent, nonspecific stimulating agent, 12-O-tetradecanoyl phorbol ester (TPA). This agent has the advantage of being a much more potent stimulating agent than the two peptides. The kinetics of response to TPA are altered slightly (relative to the response to the peptides) but in most other respects, TPA mimics the chemotactic peptides very closely. The findings made in these studies are summarized briefly in the following paragraphs.

Kinetics of stimulated adherence

Figure 1 shows the typical adherence response of the Walker cells in the nylon fiber assay when stimulated with either FMLP or TPA. With both agents, the response is rapid and transient. By 30 min after treatment the cells no longer demonstrate any increased adhesiveness over baseline values. When the two stimuli are compared, it can be seen that the response to TPA develops more slowly than the response to FMLP. It can also be seen that, although the response to TPA develops more slowly, TPA ultimately induces a greater response than FMLP.

The differences in response to the two agents is seen dramatically when plastic culture dishes are used as the substrate (Figure 2). In this assay FMLP induces a barely perceptible response while up to 60% of the cells respond to TPA. Again in this assay, the response to TPA develops after a short lag period and is transient. Kinetics of response very similar to those shown in Figure 2 are also seen when collagen matrices or monolayers of endothelial cells are used as the substrate (19).

A number of other tumor cells and normal cell types have been examined for responsiveness to the chemotactic peptides and TPA. Included in this group are three cell lines derived from a murine fibrosarcoma, two human mylogenous lines, normal rat, and mouse fibroblasts as well as rat peritoneal leukocytes. The findings made with these cells are summarized in Table 1. It can be seen that there were significant differences in the response patterns of the murine fibrosarcoma lines. The high malignant line responded to both of the chemotactic peptides and to TPA while the low malignant line was unresponsive. In this respect, the low

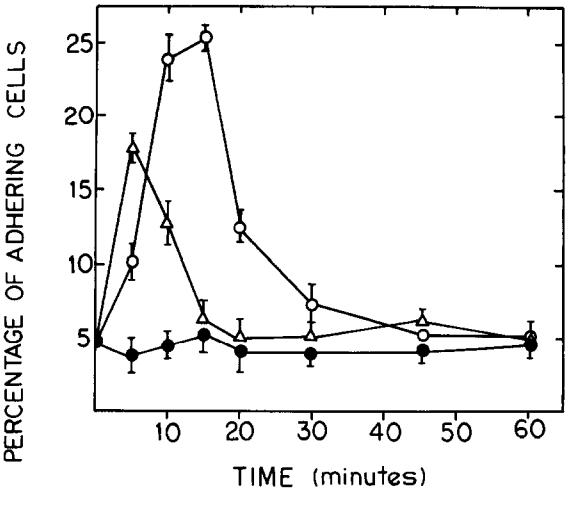

Figure 1. Adherence of Walker 256 carcinosarcoma cells to nylon fibers. The assay was performed as described previously (18, 19). Cells were maintained in suspension until added to the prewashed columns. The added cells were allowed to remain in contact with the nylon fibers for 3 min, following which the nonadherent cells were washed through with excess PBS. Shown is the mean (± standard error) of the added cells which adhered (●—●, control cells; △—△, FMLP-treated; ○—○, TPA-treated).

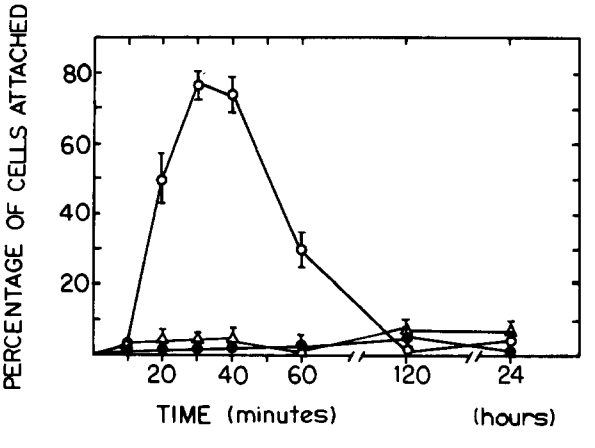

Figure 2. Adherence of Walker 256 carcinosarcoma cells to plastic culure dishes. The assay was performed as described previously (19). Cells were treated and added to the dishes at time zero. At various times later the nonadherent cells were removed. Shown is the mean (± standard error) of the added cells which adhered (●—●, control cells; △—△, FMLP-treated; ○—○, TPA-treated).

Table 1. Responsiveness of various cell types to chemotactic factors and TPA in adherence assays.

Cell type	Stimulating agent		
	C5a-derived chemotactic peptide[a]	FMLP[a]	TPA[b]
Walker 256 carcinosarcoma	+[d]	+	+
murine fibrosarcoma (parent)	ND[e]	ND	+
clone 1 fibrosarcoma[c]	+	+	+
clone 6 fibrosarcoma	–[f]	–	–
CEM (T lymphoid)	ND	ND	+
K562 (myeloblast)	ND	ND	+
Normal rat fibroblast	–	–	–
rat peritoneal leukocyte	ND	ND	+
normal mouse fibroblast	ND	ND	–

[a] See reference 18 for details
[b] See reference 19 for details
[c] The responses of the clone 1 and clone 6 fibrosarcoma cells to the C5a-derived peptide and to FMLP are unpublished. The response to TPA was reported in reference 19
[d] + indicates a statistically significant response
[e] ND (Not Done) indicates that the test has not been done
[f] – indicates no statistically significant response

malignant fibrosarcoma cells are very similar to normal fibroblasts which were also nonresponsive. This was not surprising because with regard to a number of other characteristics as well, the low malignant cells seem to be very similar to normal fibroblasts (17, 24, 37–39). On the other hand, the rat peritoneal leukocytes and the two mylogenous cell lines did become hyperadherent when stimulated with TPA. A complete description of these findings can be found in our recent reports (18, 19).

Several other investigators have also reported adherence responses in various cell types to TPA and other phorbol diesters. Among the cell types shown to respond are several malignant and nonmalignant lymphoblastoid lines, a number of erythroleukemic cell lines and normal chondrocytes (40–43). As in our studies, normal fibroblasts were found not to be responsive (41).

It should be noted that not all responsive cell lines demonstrate the same kinetics as the Walker carcinosarcoma cells. For example, while the response of the Walker cells is rapid and transient, several cell lines which normally attach and grow as adherent monolayer cells attach to the substrate much more rapidly in the presence of TPA, but then do not detach (18, 41). Other cell types which normally grow in suspension attach very slowly (effects are first seen after 1–4 hr) but then remain attached indefinitely (40, 42, 43). Our interest has been in the immediate response demonstrated by the Walker cells and other cell types, since this rapid response is similar to what is observed after stimulation with chemotactic peptides. The slow but sustained response seen in some cells may be mediated through an entirely unrelated mechanism. This would not be surprising, since TPA has such a wide variety of effects on so many cell types (44).

Physiological basis for stimulated adherence

What are the physiological events which occur in the stimulated cells and lead to the hyperadherence response? This question is being pursued actively, and it is hoped that the answer will provide the mechanism not only of the hyperadherence response but of the other responses to stimulating agents as well. While a definitive answer can not be provided at the present time, it can be postulated (based on indirect evidence) that stimulation of the tumor cells with either the chemotactic peptides or with TPA leads to a rapid activation of phospholipid metabolism. As a result of this, arachidonic acid is released and is then available for metabolism through the lipoxygenase pathway to produce the bioactive intermediates which actually mediate the response. What is the evidence in support of this? As shown in Table 2, a variety of inhibitors which are known to block either the activation of the phospholipase or the metabolism of arachidonic acid through the lipoxygenase pathway strongly inhibit either FMLP or TPA-induced adherence. These include p-bromophenacyl bromide, nordihydroguaiaretic acid and 5, 8, 11, 14-eicosatetraynoic acid. On the other hand, acetylsalicylic acid which has no anti-lipoxygenase activity but does block cychlooxygenase activity is ineffective. It can also be seen in Table 2 that indomethacin, which has

Table 2. Effects of various agents on FMLP and TPA-induced adherence of Walker cells.[a]

Agent[b]	Percent inhibition FMLP	TPA
1×10^{-5}M p-bromophenacyl bromide	98±3	80±1
1×10^{-5}M nordihydroguaiaretic acid	99±5	99±8
1×10^{-4}M 5, 8, 11, 14-eicosatetraynoid acid	ND[c]	49±3
1×10^{-3}M acetylsalicylic acid	2±1	4±5
1×10^{-6}M indomethacin	ND	−20±2
1×10^{-5}M indomethacin	ND	−17±3
1×10^{-4}M indomethacin	5±4	1 × 1
1×10^{-3}M indomethacin	50±6	48±1

[a] The nylon fiber assay was used with FMLP, and the plastic culture dish assay was used with TPA. They were performed as described previously (18, 19). Each inhibitor was made up to the desired concentration and added to the cell suspension. The cells were pretreated for 30 min and then tested in the normal manner. In order to show enhancement of induced adherence by pretreatment of the cells with low doses of indomethacin, a 16 hr preincubation was done

[b] Inhibition of TPA-induced adherence by p-bromophenacyl bromide, nordihydroguaiaretic acid, indomethacin (1×10^{-3} – 1×10^{-4}M) and acetylsalicylic acid was reported previously (19). The other data in this table were previously unpublished

[c] Not done

some inhibitory activity at very high concentrations, (10^{-3}M) actually potentiates induced adherence at lower concentrations (10^{-5}–10^{-6}M). This strongly suggests that lipoxygenase intermediates are actively involved in the stimulated adherence response.

The same inhibitors which we have used to modulate stimulated adherence in the Walker tumor cells have been used in previous studies with leukocytes. The effects of these agents on stimulated leukocyte functions closely parallel the effects on the tumor cells. For example, p-bromophenacyl bromide, nordihydroguaiaretic acid and 5, 8, 11, 14-eicosatetraynoic acid are potent inhibitors of chemotactic factor-induced aggregation, lysosomal enzyme release and superoxide generation by leukocytes (45–47). Indomethacin and acetylsalicylic acid are much less effective (45–47).

Inhibitor data by itself cannot prove or disprove an hypothesis. What is needed are direct qualitative and quantitative studies showing the spectrum of lipoxygenase products made in control and stimulated cells, and, hopefully, direct evidence showing that one or more of these products can duplicate the effects produced by chemotactic factor or TPA stimulation. Both types of investigation are currently underway in our laboratory. While the direct evidence with the tumor cells must await the outcome of ongoing studies, studies in a number of animal cell models have shown that TPA does stimulate phospholipid metabolism (48–50). In bovine lymphocyte cultures, increased incorporation of precursers into phospholipids can be detected as early as 20 min after stimulation (48). Presumably the increase incorporation is associated with elevated rates of phospholipid turnover, although increased total synthesis is also thought to occur. Analogues of TPA such a 4B-phorbol-12, 13-didecanoate and 4B-phorbol-12, 13-dibenzoate which also stimulate Walker cell adherence (19) likewise induce phospholipid metabolism in the bovine lymphocyte cultures while analogues which are nonreactive in the adherence assay (4B-phorbol-12, 13-diacetate and 4B-phorbol) do not. The phospholipid synthesis stimulated by TPA can be inhibited by 5, 8, 11, 14-eicosatetraynoic acid but not by indomethacin. This inhibition can be reversed by arachidonic acid but not by any of several cychlooxygenase products tested nor by other fatty acids. This datum strongly suggests the functioning of a lipoxygenase product in this system.

In dog kidney cells (50) TPA stimulation causes a deacylation of arachidonic acid-containing phospholipids. In this system most of the released arachidonic acid is found 14 hr later in a variety of prostaglandin compounds. What lipoxygenase products are also produced in this system have not been reported.

The chemotactic peptides have not been studied in as wide a variety of cells as TPA but have been extensively studied in leukocytes. In leukocytes, stimulation leads to the liberation of arachidonic acid from membrane phospholipids. The released arachidonic acid is then transformed by lipoxygenation and subsequent enzymic reaction to a variety of lipid mediators of the diverse responses (51–55).

In summary, then, the metabolic events postu-

lated here to underlie the tumor cell hyperadherence response to the chemotactic peptides and to TPA have been shown to occur in other cell types upon stimulation with these agents. Because of the similarities between the tumor adherence model and several other models (particularly the leukocyte model), it seems reasonable to postulate that similar biochemical events are occurring in the tumor cells. To reiterate what was said previously, however, the conclusion that these same metabolic events underlie the tumor cell adherence response to chemotactic peptides and TPA must be considered tentative until the direct evidence in the tumor cell system itself is available.

Even granting the tentative conclusion that activation of phospholipid metabolism underlies the tumor cell response still does not tell us how the transient hyperadherence comes about. A number of possible mechanisms exist. It is known that the chemotactically responsive tumor cells bind concanavalin-A (Con-A) and that Con-A cap formation (which occurs with a low frequency in control cells) is decreased in chemotactic factor-treated cells (56). Cap formation results from a coalescing of the surface receptors for Con-A. decreased cap formation in the treated cells suggests a rearrangement of the cell surface structures as a result of treatment with the chemotactic factor. The percentage of cells which demonstrate cap formation after treatment is similar to the percentage which demonstrate hyperadherence. The dose response curve for chemotactic factor-induced decrease in cap formation is the same as for adherence. We have speculated that a similar rearrangement of surface adherence molecules may also occur, perhaps leading to the accumulation of enough adherence molecules at particular membrane sites, to facilitate the induction of cell-to-substrate binding.

On the other hand, it may not be a localization of adherence molecules but a general change in membrane properties which alters the adhesiveness of the cells. Possibly the lysophosphatides remaining in the membrane when arachidonic acid is split out or the newly formed phospholipids which are generated upon reacylation confer new adherence characteristics on the cells. The lipoxygenase intermediates of arachidonic acid themselves can be found in the membranes of stimulated cells (57, 58). If either of these suggestions is correct, it would imply that the altered adhesiveness due to stimulation is primarily a membrane event. A similar conclusion was reached by O'Flaherty et al. (59) who suggested that with leukocytes, cell-to-cell aggregation is primarily a membrane event.

In contrast, stimulated adherence may be the result of effects that are felt primarily at the cytoskeletin. This would not be too surprising, since an intact cytoskeletin is required for the normal adherence and spreading of cells in culture (60). We have shown previously that stimulated adherence can be totally inhibited by either colchicine or cytochalasin B (19). Obviously, much more work needs to be done before any of these ideas are validated.

Chemotactic responses and metastases

Only a very small percentage of the tumor cells which enter the circulation survive passage through the vasculature and successfully lodge at secondary sites where metastatic tumors ultimately develop. It is our working hypothesis that the response of circulating tumor cells to chemotactic activation influences the ability of responsive cells to localize at the secondary sites. Activation of the circulating cells could influence their ability to localize in either of two ways. First, the systemic activation of a large number of tumor cells could increase the number of cells which ultimately localize successfully. Second, the extravascular production of an activating factor could induce the localization of cells near the site of its production (thereby influencing the distribution of the cells).

What experimental evidence is available to support these ideas? The first direct evidence supporting the concept of chemotactic localization of tumor cells was provided by Ozaki et al. (61) and Hayashi et al. (4). They were able to induce the intradermal localization of the chemotactically responsive AH109A hepatoma cells by intradermal injection of the tissue-derived chemotactic factor into rats bearing circulating tumor cells. Histological examination of the skin sites within 24 hr

revealed a number of tumor cells sticking to the endothelial cells of the venules and some tumor cells already in the process of emigrating through the walls of the vessels. By 72 hr, the extravascular tumor cells had increased in number, and mitotic figures could be seen. At periods longer than this, widespread invasion of the underlying connective tissue and muscles could be seen. Similar findings to these in rats were obtained in mice injected with the C1498 myeloid leukemia cells (also shown to be chemotactically response in vitro). In animals not injected with tumor cells, injection of the chemotactic factors induced a mild edematous change and the accumulation of a few neutrophils. In contrast to these results, rats injected with either permeability-increasing factors or with leukocyte chemotactic factors failed to develop metastatic tumors at the site of injection. On the basis of these results it was concluded that the accumulation of tumor cells at the sites of chemotactic factor injection is a specific response to the chemotactic factor and not a nonspecific consequence of permeability alterations or nonspecific inflammatory processes.

In a second experimental model we demonstrated the localization of chemotactically responsive tumor cells in the peritoneal mesenteries following intravenous injection of the cells and intraperitoneal injection of the C5a-derived tumor chemotactic peptide (62). The advantage of this model is that no trauma is done to the tissue in which the metastatic tumors ultimately develop. The developing tumors could be seen histologically surrounding the capillaries and small venules rather than on the surface of the mesenteries. When radiolabeled tumor cells were used, it could be shown that the intraperitoneal injection of the chemotactic factor induced an increased localization of these cells rather than an increased growth of tumor cells already present (63).

These two model systems clearly indicate that the distribution of metastasizing tumor cells (if not the actual number) can be influenced by the injection of chemotactic factors into experimental animals. The problem with both models is that only a very small percentage of the injected cells end up at the target sites. This is probably due to the fact that most of the injected cells are rapidly cleared from the circulation and end up in organs such as the lung. Furthermore, a very high percentage of cells are rapidly destroyed leaving only a few to respond to stimulation. Finally, the injection of small molecular weight factors into extravascular sites can be expected to rapidly diffuse from the sites. We are currently working on ways to overcome these limitations. Studies are in progress to determine if it is possible to influence the distribution of circulating tumor cells by systemically activating the cells. This approach is theoretically very similar to that used by O'Flaherty et al. (64, 65) to demonstrate the effects of systemic activation of leukocytes on their distribution.

The exact mechanism through which circulating tumor cells become localized in vivo is not known. Adherence of the cells to the vasculature wall is likely to be one of the initial events. The classical studies by Wood (66) using the rabbit ear chamber to examine tumor cell invasion in vivo documented the role of adherence in this process very elegantly. After viable tumor cells were injected into the vessel 'upstream' from the placement of the chamber, it was found that individual tumor cells became attached to the endothelial cells in a seemingly random fashion. Although most of the cells that attached were quickly dislodged and carried away, some of the cells became firmly adherent to the endothelial cell layer. Fibrin and platelet-containing thrombi were frequently seen in conjunction with the firmly adherent cells. Later, active migration was seen to occur in these cells leading to extravasation of the tumor cells into the extravascular tissue. These elegant in vivo studies are supported by several recent reports showing correlations between increased tumor cell adherence in vitro and increased metastatic capacity after intravenous injection (30–35).

How chemotactic factors work to directly influence tumor cell localization in vivo is not known, but since these agents dramatically increase cell adhesiveness, it is tempting to speculate that this response is involved. It is interesting that activated cells demonstrate increased adhesiveness to a variety of substrates, including monolayers of endothelial cells (19). Once the cells have become adherent to the endothelial cell layer in vivo,

additional factors may come into play. Perhaps, as suggested by Wood (66), fibrin and platelet deposits form at the site stabilizing the adherence. Active cell motility (67) and matrix degradation (68, 69) along with cell proliferation could occur. These processes might themselves be subject to stimulation by chemotactic factors or other activating agents. Furthermore, these processes might be triggered directly by the adherence of the tumor cells to the substrate. In any event, the sum total of these responses could lead to the successful invasion and growth of the tumor cells at the secondary, metastatic site.

Much more work needs to be done before the full implication of chemotactic responses in tumor cells is known. Current efforts in our laboratory and in other laboratories are aimed at the following: 1) identifying and characterizing naturally occurring tumor cell chemotactic factors and identifying the various in vitro responses to chemotactic factors; 2) demonstrating in vivo responsiveness to these factors; 3) identifying variability among tumor cell populations in their responsiveness to chemotactic factors and correlating responsiveness with metastatic capability and 4) delineating the biological mechanism underlying the responses and identifying ways to modulate them.

New information with regard to several of these areas is likely to be forthcoming. For example, some of the ongoing studies in our laboratory are directed at modulating the response of the tumor cells to chemotactic factors. These studies show that the hyperadherence response in the tumor cells can be inhibited by prostaglandins with antiaggregatory activity (70). Perhaps it will be possible to modulate the metastatic process with these agents in much the same way that the inflammatory process can be modulated (71).

Conclusions and future work

It is clear that tumor cells are subject to activation by chemotactic factors and similar substances in vitro. Under appropriate conditions, in vivo responses to these factors can be demonstrated. We suggest that the ability of tumor cells to respond to such agents in vivo may contribute to their metastatic ability. This is not to suggest that all tumor cells respond to these agents or that the metastatic ability of tumor cells is solely controlled by responses to these factors. If anything can be said about tumor cells, it is that they are extremely diverse, and no one mechanism is likely to be relevant to all tumor cells. None the less, the ability to respond to activating agents may be an important tool is the overall repertoire of tumor cells influencing their metastatic behavior.

Finally, the significance of the chemotactic response in tumor cells may extend beyond the field of metastasis. The activation process in tumor cells has many features in common with activation processes in other cells by specific agonists. The tumor cells may provide a unique model with which to probe the underlying mechanism of stimulus-coupled events in cells in general. The tumor cells have certain advantages over a number of other cell types. Among these advantages are rapid, continuous growth under controlled conditions in vitro, the availability of large numbers of cells at any one time, and the availability of subpopulations with unique characteristics. Obviously, each of these could be a tremendous advantage. We may ultimately come full cycle to the point where we acknowledge the similarities (rather than the differences) between neoplastic cells and their normal counterparts.

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