Chemotactic Factor-Induced Adherence of Tumor Cells

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Abstract

Two peptides which have previously been shown to induce chemotactic motility in a number of tumor cells were tested for their ability to alter the adhesiveness of Walker Carcinosarcoma cells (A chemotactically-responsive rat tumor) and normal rat fibroblasts (which have previously been shown to be chemotactically nonresponsive). Adherence of the tumor cells to nylon fibers was increased in a dose-dependent manner by the two active peptides. Adherence of the fibroblasts was not increased. Nonchemotactic peptide analogues of the two active peptides did not alter the adherence of either cell type. The increased adhesiveness to foreign surfaces may contribute to the chemotactic response.

Introduction

Several lines of investigation over the past decade have indicated that chemotactic motility occurs in a variety of cell types and that this phenomenon contributes to such diverse biological processes as inflammation, wound healing and tumor cell metastasis (Ward et al. 1965; Zigmond and Hirsch, 1973; Postlethwaite et al. 1976; Postlethwaite et al. 1978; Hayashi et al. 1970; Orr et al. 1978). While the basis for the chemotactic response in leukocytes is not yet well understood, very little (other than the fact that it occurs) is known about the responce in non-leukocyte cells. For the past several years we have been examing the role of chemotactic motility in the process of tumor cell metastasis. We report here that factors which are known to induce chemotactic motility in these cells simultaneously cause increased adherence of these cells to foreign surfaces. Since adherence to foreign surfaces is known to be essential for cell motility to occur, the ability of the chemotactic factors to induce increased adherence in responsive cells may underlie the chemotactic response in these cells.

Materials and Methods

Cells. The tumor cells used in these experiments were Walker carcinosarcoma cells maintained in the ascites form in male Sprague/Dawley rats. The routine handling of the cells and the

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preparation of the cells for assay have been described in our recent report (Orr et al. 1978). Normal rat fibroblasts obtained from the lungs of healthy rats and maintained in monolayer culture were used as control cells. For assay purposes the cells maintained in culture were harvested by trypsinization and then kept on an orbital shaker in growth medium (RPMI-1640 + 10% fetal calf serum) for a period of 4 hours prior to use.

Analytical Methods. Adherence was measured using the nylon fiber assay (MacGregor et al. 1974). One hundred mg of scrubbed nylon fibers (Associated Biomedic Systems; Buffalo, New York) were weighed in an analytical balance, packed into plastic 10cc syringes (to the 3cc mark) equipped with 2-way stopcocks and placed vertically in a test tube rack. After prewashing the columns with Hank's balanced salt solution (HBSS), 1-ml samples of cells treated with either the chemotactic factor or an appropriate control were added to the columns. After allowing the cells to adhere for 3 minutes the stopcocks were opened and the nonadherent cells washed through with 20 ml of phosphate buffered saline. The number of cells washed out of each column was counted and the percentage of adhering cells determined. All cell counts were performed on a Coulter counter ZBI (Coulter Diagnostics, Hialeah, Florida).

Chemotactic factors used in these experiments include the C5-derived tumor cell chemotactic peptide prepared by trypsinization of the C5-leukotactic peptide (Orr et al. 1978) and the chemotactic tripeptide, N-formyl-methionyl-leucyl-phenylalanine (f-met-leu-phe) which was obtained from Sigma Chemical Company (St. Louis, Missouri). Non chemotactic analogues which were used include the leukotactic C5 chemotactic peptide, the dipeptide, N-formyl-methionyl-phenylalanine (f-met-phe) obtained from Sigma Chemical Company and the tripeptide, N-formyl-methionyl-methionyl-phenylalanine (f-met-met-phe) obtained form Miles Ltd. (Yeda, Israel).

Results and Discussion

Table 1 shows the effects of the two chemotactic factors on the adherence of Walker carcinosarcoma cells to nylon fibers. It can be seen that both factors increased the adherence values significantly over baseline values in a dose-dependent fashion. These doses of the chemotactic factors which were effective in the adherence assay were the same doses which we have previously shown to be effective in stimulating chemotactic motility and cell swelling (Orr et al. 1978; Wass et al. 1980).

In contrast to these results, the peptide analogues of f-met-leu-phe did not stimulate increased adherence when tested over the same concentration range (10^{-6}-10^{-12}M). Likewise the untrypsinized C5-derived leukotactic peptide stimulated only minimal adherence when tested over the same range as the active tumor cell chemotactic peptide (0.1-5 ED_{50}). The minimal degree of adherence stimu-

lated by the untrypsinized C5-derived peptide is not surprising since previous studies have shown that this peptide induces only minimal chemotactic activity in the same tumor cells (Romualdez et al. 1976; Orr et al. 1978). Other studies have shown that the f-met-leu-phe analogues used in these experiments were also ineffective as chemotactic factors for the tumor cells and did not stimulate cell swelling (unpublished observation). These data are shown in Table 1.

Table 1. Effect of chemotactic factors on the adherence of Walker carcinosarcoma cells and rat fibroblasts

Chemotactic factor	Percent adherence to nylon wool (± s.e.m.)[a]	
	Walker cells	Normal fibroblasts
HBSS	26 ± 4	20 ± 2
0.1 ED_{50} C5-tumortactic peptide	30 ± 1	------
0.5 ED_{50} C5-tumortactic peptide	38 ± 6	22 ± 3
1.0 ED_{50} C5-tumortactic peptide	43 ± 7	19 ± 5
5.0 ED_{50} C5-tumortactic peptide	39 ± 7	------
0.1 ED_{50} C5-leukotactic peptide[b]	27 ± 4	22 ± 3
1.0 ED_{50} C5-leukotactic peptide	33 ± 7	23 ± 5
50 ED_{50} C5-leukotactic peptide	30 ± 5	22 ± 1
10^{-14}M f-met-leu-phe	24 ± 2	------
10^{-12}M f-met-leu-phe	39 ± 6	------
10^{-10}M f-met-leu-phe	42 ± 6	19 ± 7
10^{-8}M f-met-leu-phe	48 ± 3	21 ± 3
10^{-6}M f-met-leu-phe	38 ± 7	------
10^{-12}M f-met-phe	25 ± 4	------
10^{-10}M f-met-phe	27 ± 3	------
10^{-8}M f-met-phe	27 ± 4	------
10^{-6}M f-met-phe	24 ± 4	------
10^{-12}M f-met-met-phe	23 ± 2	------
10^{-10}M f-met-met-phe	29 ± 7	------
10^{-8}M f-met-met-phe	30 ± 3	------
10^{-6}M f-met-met-phe	27 ± 4	------

[a]The values shown are the averages of 3 experiments. In each experiment triplicate columns were used.

[b]The C5-leukotactic peptide is the precurser peptide from which the tumor cell chemotactic factor is derived. Its preparation and the preparation of the tumor cell chemotactic factor are described in our recent publication (Orr et al. 1978).

In other studies (data not shown) 10^{-3}M 2-deoxyglucose was added to the cell suspension at the time that the cells were used in the adherence assay. For this study HBSS was made up without glucose. Under these conditions adherence to nylon fibers in the absence of chemotactic factor was similar to what was observed in complete HBSS (23 ± 5% vs 27 ± 7%). However, when 10^{-6}M f-met-leu-phe was simultaneously added, the increased adherence was greatly reduced relative to what was seen in complete HBSS (35 ± 4% vs 48 ± 5%). This observation, indicating that chemotactic factor-induced adherence is an energy-dependent process, is similar to what was observed in the cell swelling and chemotactic assays (Wass et al. 1980). In contrast to the results observed with the Walker carcinosarcoma cells, it can be seen in Table I that the normal rat fibroblasts did not respond to the chemotactic factors in the adherence assay. These cells have previously been shown to be nonresponsive to the same factors in the Boyden chamber assay for chemotaxis (Orr et al. 1981).

It is well established that motility of mammalian cells is greatly influenced by the substrate on which they are migrating and that if the cells are not able to form the proper contacts with the substratum, motility will be inhibited (Carter 1967; Vasiliev and Gelfand 1975). It is significant, therefore, that we have demonstrated in these experiments that chemotactic factors increase the foreign surface adhesiveness in responding cells. It may be that the increased adhesiveness induced by these factors underlies the chemotactic response. This may also explain why chemotactic factors tend to increase the random motility of responsive cells in addition to stimulating chemotactic motility.

The ability of neoplastic cells to respond to chemotactic factors derived from C5 may contribute to their ability to localize at sites of inflammation since it has been shown how the C5-derived tumor cell chemotactic factor can be generated by leukocyte proteases (Orr et al. 1979) and by proteases from other tissues (Romualdez et al. 1976). It is interesting in this light that the normal fibroblasts did not respond - either in the adherence assay (shown here) or in the Boyden chamber assay for chemotaxis (Orr et al. 1981). This would indicate some specificity in the chemotactic response, at least to the complement-derived factors. This is not to suggest that normal fibroblasts are not chemotactically responsive. Postlethwaite et al. (1976; 1978) have clearly shown that they are responsive to a number of factors. It is just that the agents to which they respond are different.

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