Antileukotactic Properties of Tumor Cells

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ABSTRACT A chemotactic factor inactivator (CFI) has been found in extracts of Walker and Novikoff tumor cells maintained in rats. The CFI directly inactivates the bacterial chemotactic factor as well as the leukotactic activity (for both neutrophils and monocytes) associated with C3 and C5 fragments and with culture fluids of lectin-stimulated lymphoid cells. The inactivation of the bacterial chemotactic factor is temperature and pH dependent. Subcellular fractionation procedures indicate that CFI is largely associated with the microsomal and cytosol fractions of tumor cells. CFI activity is also found in rat neutrophils, alveolar macrophages, and in extracts of liver, spleen, and kidney from normal animals. CFI derived from normal tissues also directly inactivates the bacterial chemotactic factor and has the ability to inactivate chemotactic activity associated with C3 and C5 fragments. A feature of the tumor-associated CFI is its presence in ascitic fluids of animals bearing tumor cells and the relative absence of any CFI activity in acute inflammatory exudates. The finding of the tumor-associated CFI may explain, at least in part, the tendency of malignant tumor cells to suppress cellular inflammatory reactions.

INTRODUCTION

There is abundant evidence to indicate that cellular inflammatory responses are defective in humans who bear malignancies unrelated to the hematopoetic system (1–4). The extent to which a causal relationship exists between defective cellular inflammatory responses and ineffective control of malignant tumors by the host is not presently known. Since lymphocytes and monocytes participate in cytotoxic reactions directed towards tumor cells (reviewed, 5), it is not difficult to understand how the inability to accumulate these leukocytes in the vicinity of tumor cells might lead to difficulties in the control of tumors. The well known observations that adenocarcinomas of the breast carry a much better prognosis if associated with infiltrates of lymphocytes and monocytes, contrasted with similar tumors that incite little or no inflammatory cellular response, would seem to emphasize the importance of the inflammatory response in the control of tumors (6).

Although it seems widely agreed that a significant number of patients with malignancies fail to express inflammatory reactions of the delayed hypersensitivity type, the belief that this defect is limited to the expression of delayed hypersensitivity reactions has been questioned inasmuch as agents (such as crotin oil) that do not demonstrably require an immunological mechanism for the expression of their inflammation-producing activity also fail to incite cellular inflammatory reactions in the skin of tumor-bearing patients (7). These observations would tend to suggest a defect involving more than the immune apparatus. Recently, observations have been published describing the ability of tumor cells to "repel" macrophages (8). In the same studies, it was suggested that tumor cells fail to incite cellular inflammatory responses in skin when compared with nontumor cells. Similar observations were reported several years ago by other investigators who used a cotton thread implanted in vivo into a bed of connective tissue containing, or devoid of, transplantable tumor cells. Quite consistently, the cellular inflammatory response to the foreign body was suppressed in the presence of malignant tumor cells (9). All of these findings have suggested that malignant cells somehow prevent the inflammatory cellular response and accumulation of leukocytes.

In the studies to be reported in this paper, a leukotactic defect due to a tumor cell product is described. Tumor cells contain a chemotactic factor inactivator (CFI) [1] that inactivates C3, C5, bacterial, and lymphokine-associated leukotactic mediators. Ascitic fluids in tumor-bearing rats also contain a CFI. A similar in-

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[1] *Abbreviation used in this paper:* CFI, chemotactic factor inactivator.

activator can be isolated from many normal tissues. These studies may explain why cellular inflammatory responses are defective in the face of malignant tumors and why malignant tumors fail to incite cellular inflammatory responses.

METHODS

Leukotactic assays. These were performed in vitro with modified Boyden chambers containing micropore filters of 0.65 μm. In each experiment assays were carried out using both rabbit and rat neutrophils obtained by intraperitoneal injection of glycogen (10). The results were similar regardless of the species of cell employed. The leukotactic assay has been described in detail elsewhere (11). Leukocytes were suspended in Hanks' medium containing 0.1% bovine serum albumin.

Leukotactic factors. The bacterial chemotactic factor present in a culture medium (50 μl) after overnight growth of *Escherichia coli* was employed (12). This factor is heat stable and resists boiling. Where various extracts or fluids were tested for their ability to inhibit the bacterial chemotactic factor, the usual volume of test material was added to 50 μl bacterial factor, incubated for 30 min at 37°C at pH 7.3 (0.01 M Tris buffer), and then the residual chemotactic activity assessed by diluting the mixture to 1.0 ml with Hanks' medium. Details are given where this protocol was modified.

The chemotactic factors derived from the third (C3) and the fifth (C5) components of complement were also used in some experiments. These were obtained by appropriate trypsin treatment of purified human C3 and C5 (13). In a given experiment, the equivalent of 20 μg intact C3 and C5 was used.

Tumor cells. Walker carcinosarcoma cells and Novikoff hepatoma cells, kindly supplied by Dr. I. Wodinsky (Arthur D. Little, Inc., Cambridge, Mass.), were maintained in the ascitic form in outbred Long-Evans rats, with serial passage, using 5×10^6 cells approximately every 7 days. When cell disruption techniques were employed, a "Biosonic" probe device (Bronwill Scientific, Rochester, N. Y.) was used. Sonication time and intensity were adjusted such that cell disruption occurred, but many nuclei remained intact (as monitored by light microscopy). Tissue homogenates were prepared according to previous techniques (14).

TABLE I

CFI Extractable from Walker and Novikoff Tumors

Tumor cells	CFI activity*
	% inactivation
Walker	62
	63
Novikoff	100
	78

* Present in 50 μl extract from tumor cells suspended at 10^7 cells/ml. Each number represents extracts from cells harvested from a different animal. Chemotactic values of positive (chemotactic factor) and negative (culture medium) controls for this experiment were 150 and 10, respectively.

TABLE II

CFI Activity as a Function of Temperature of Incubation

Temperature	CFI activity* Exp A	Exp B
°C	*% inactivation*	
0	2	6
25	59	32
37	65	56

* Extract of Walker tumor cells. In each experiment an equivalent of 5×10^5 tumor cells was used. Incubation at the designated temperature was for 30 min followed by dilution in Hanks' medium and assay for residual chemotactic activity. The numbers under A and B represent percent inactivation of bacterial chemotactic activity. Positive and negative chemotactic counts of controls for this experiment were 300 and 40, respectively.

Leukocytes. In some experiments sonicates of rat neutrophils and alveolar macrophages were used. The former cells were obtained 5–6 h after intraperitoneal injection of 0.1% glycogen in saline. Alveolar macrophages were obtained as a pool from 5–10 rats after exsanguination from the inferior vena cava and wash-out of the tracheal-bronchical tree with saline (15).

Subcellular fractionation. Disruption of tumor cells suspended in Hanks' medium was carried out by sonication, as described above, followed by sedimentation of cell homogenates in a discontinuous gradient of sucrose, as described by de Duve (16). The success of the subcellular fractionation was assessed by partitioning of enzymes as described elsewhere (17). Both enzyme assays and electron-microscopic analysis indicated the anticipated separation of subcellular constitutents.

Monocyte chemotaxis. Monocytic leukocytes were obtained from oil-induced peritoneal exudates in rats. For chemotactic testing, cells were suspended in minimal essential medium (MEM) containing 10% homologous serum. Micropore filters of 5-nm pore size were used. Factors employed were the C3 and C5 fragments and culture fluids (diluted 1:5) from rat lymphoid cells stimulated with conconavalin A. Details are given in a recent report (13).

RESULTS

CFI activity in tumor cells.[2] Crude homogenates of either Walker carcinosarcoma cells or Novikoff hepatoma cells, harvested from ascitic fluids of Long-Evans rats, demonstrated inhibitory activity for the bacterial chemotactic factor (Table I). From each of four different rats, the extracts from cell equivalents of 5×10^5 tumor cells inactivated between 62–100% of the chemotactic activity. The ability of Walker cell extract to inactivate the bacterial chemotactic factor was studied as a function of the temperature of incubation. In two

[2] In this and subsequent sections reference is made to the CFI and chemotactic factor inhibitor. As will be shown, the inhibitor is in fact functioning as an inactivator. The terms are, thus, interchangeable.

TABLE III
Specificity of CFI from Various Rat Tissues

Tissue source of CFI*	Inactivation of chemotactic factors		
	C3 fragment	C5 fragment	Bacterial factor
	% inactivation		
Walker tumor cells	80	100	88
Liver	45	80	90
Spleen	70	100	64
Neutrophils	70	100	88

* The equivalent of 5×10^6 cells was used in the case of Walker cells and neutrophils, whereas the equivalent of 3 mg solid tissue was employed from liver and spleen. Chemotactic values for the C3, C5, and bacterial chemotactic factors were 265, 325, and 300, respectively, with a negative control (culture medium alone) value of 25.

different experiments the degree of inactivation of bacterial chemotactic factor was directly proportional to the temperature (Table II). At 0°C virtually no inactivation occurred, whereas maximal effect over the temperature range studied was at 37°C.

Specificity of tumor-associated CFI activity. To determine if the inactivator from Walker tumor cells was restricted to an effect on the bacterial chemotactic factor, an extract from the tumor cells was incubated with the complement-dependent C3 and C5 chemotactic fragments and the amount of inactivation of the chemotactic activity determined. As shown in Table III, there was a consistent inactivation of all three chemotactic factors by the tumor cell extract. This is similar to the spectrum of activity of the CFI isolated from whole human serum (13). As will be described below, extracts of other tissues and cells also contained an inactivator of chemotactic factors.

TABLE IV
Localization of CFI in Subcellular Fractions of Walker Tumor Cells

Fraction tested*	CFI activity	
	Exp A	Exp B
	% inactivation	
Crude cell homogenate	84	65
Nuclear fraction		20
Mitochondrial fraction	2	15
Lysosomal fraction	4	35
Microsomal fraction	49	95
Cytosol fraction	49	70

* The cell equivalent of 5×10^5 cells was used and incubated with the bacterial chemotactic factor under conditions described in Methods. Positive and negative chemotactic counts of controls in this experiment were 255 and 25, respectively.

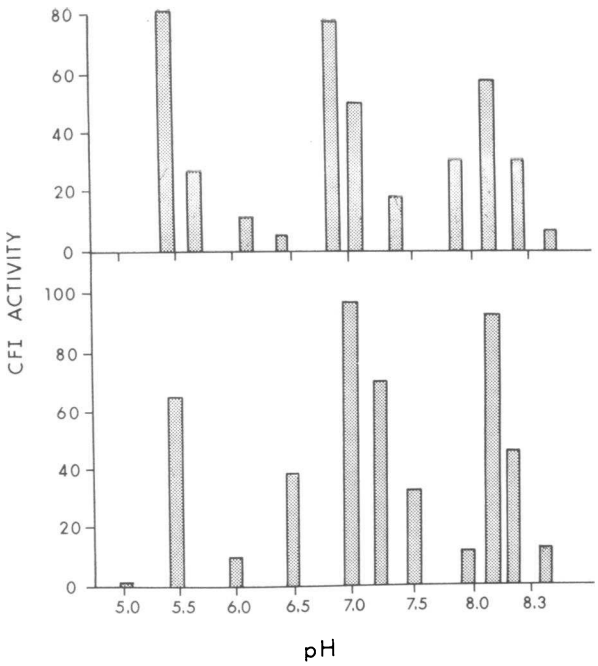

FIGURE 1 Assessment of effect of pH on CFI activity in sonicated extract of Walker tumor cells. See text for details.

Subcellular localization of CFI in Walker tumor cells. Walker tumor cells were fractionated according to standard methods (16), and each subcellular fraction was compared with the original crude cell homogenate for ability to inactivate the bacterial chemotactic factor. The subcellular fractions included preparations of nuclei, mitochondria, lysosomes, microsomes, and the cytosol fraction. β-glucuronidase was maximal in the microsomal and lysosomal fractions with half as much activity in the cytosol fraction and virtually no activity in other subcellular fractions. Glucose-6-phosphatase was present in high amounts in the microsomal fraction, and virtually none was found in other subcellular fractions. Acid phosphatase was maximal in the cytosol fraction, but approximately one-third of the same amount was found in the lysosomal fraction and half again as much in the cytosol fraction. The partitioning of enzymes represents the anticipated fractionation (16). As is evident from the data in Table IV, high degrees of inactivation of chemotactic activity occurred with the crude homogenate, the microsomal fraction, and the cytosol fraction; whereas much less inactivation of the chemotactic activity occurred when the nuclear, mitochondrial, and lysosomal fractions were used.

pH profile of tumor-associated CFI. To determine the pH optimum for inactivation of the bacterial chemotactic factor by the Walker tumor cell extract, 50 μl

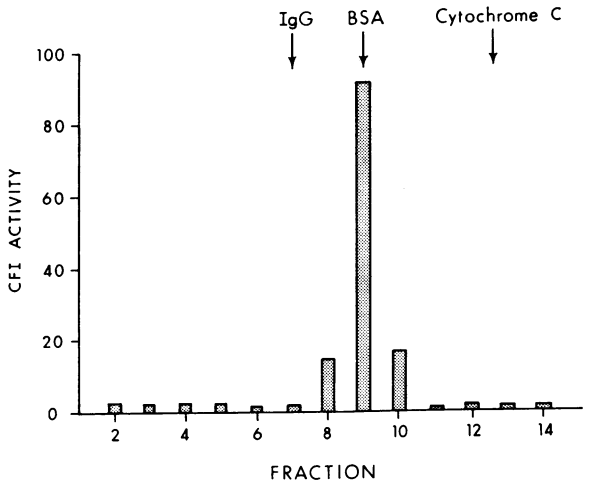

FIGURE 2 Ultracentrifugal characteristics of CFI in Walker tumor cell extract. Positions of marker proteins human IgG, bovine serum albumin (BSA), and cytochrome *c* are shown. CFI assay was performed at pH 7.3.

volumes of sonicate (equivalent to 5×10^5 tumor cells) were incubated with 50 μl bacterial chemotactic factor at 37°C for 30 min, cooled at 5°C, and followed by adjustment of pH to 7.3. The material was then diluted to 1.0 ml with Hanks' solution and residual chemotactic activity assessed. The salts used for original pH adjustment (above or below pH 7.3) included Tris-maleate buffer (18).

In Fig. 1, the results from two different experiments are shown. Three distinct zones of CFI activity were found around pH 5.5, 7.0, and 8.2. The results were consistent in two independent experiments (Fig. 1, upper and lower frames). These data suggest a heterogeneity in the tumor-associated chemotactic factor inactivator and suggest the possibility of three different inactivators.

Ultracentrifugal features of the inactivator. The Walker tumor cell extract was fractionated by sucrose density gradient ultracentrifugation, and each fraction was tested at neutral pH for inhibiting activity on the bacterial chemotactic factor. As shown in Fig. 2, the inactivator activity sedimented as a homogenous peak coincident with the bovine serum albumin marker. Although the pH studies indicate a heterogeneity in the inactivator (Fig. 1), at a neutral pH the findings in Figs. 1 and 2 suggest the action of a homogeneous substance, although physical-chemical homogeneity does not preclude heterogeneity. As will be discussed below, a CFI can be derived from rat serum. This material has a sedimentation velocity identical with the CFI extracted from Walker tumor cells.

CFI activity in tumor cells and in leukocytes. Crude cell extracts from Walker tumor cells, glycogen-induced peritoneal neutrophils, and alveolar macrophages were each compared, at three different concentrations of cell equivalents, for ability to inactivate the bacterial chemotactic factors. All cell types were found to contain an inactivator of the bacterial chemotactic factor (Table V). The findings suggested that neutrophils might be more active in this regard, but at the intermediate and highest concentrations the preparations from each cell extract inactivated greater than two-thirds of the chemotactic activity.

To rule out the possibility that the neutrophil-associated chemotactic factor inhibitor might be attributable to the inhibitor of cell motility described by Goetzl and Austen (19), it was found that if the neutrophil extract was first heated at 56°C for 1 h and then incubated with the chemotactic factor, no inactivation of the chemotactic factor occurred. However, when incubating at 37°C for 30 min the neutrophil extract with the chemotactic factor before heating at 56°C for 1 h resulted in 98% inactivation of the chemotactic factor. Heating of the bacterial factor at 56°C for 1 h had no effect on the chemotactic activity. The finding of heat lability of the CFI would seem to rule out the presence of the heat stable cell-directed inhibitor described by Goetzl and Austen (19). (The findings relative to these points are more fully elaborated upon in a subsequent Table.)

CFI activity in serum, normal tissues, and in Walker tumor cells. The findings in the preceding section, where leukocyte extracts were used, suggested the possibility that the CFI extracted from tumor cells was not a unique attribute of neoplastic cells. Sonicates of lung, kidney, liver, and spleen from normal Long-Evans rats were obtained and the tissue equivalent of 3 mg intact tissue incubated with the standard amount (50 μl) of the bacterial chemotactic factor. By way of comparison, the equivalent of 5×10^6 Novikoff tumor cells was also tested, as was the redissolved fraction of rat serum isolated fractionally with ammonium sulfate at 40 and 60% of saturation. In all cases virtually all

TABLE V
CFI Activity in Leukocyte Extracts

Extract tested	CFI activity*		
	3×10^3‡	3×10^4‡	3×10^5‡
	% inactivation		
Walker tumor cells	7.5	65	73
Neutrophils	65	70	88
Alveolar macrophages	10	70	80

* Positive and negative counts of controls for this experiment were 270 and 20, respectively.
‡Number of cells used.

TABLE VI

CFI Activity Extractable from Normal Rat Tissues and Tumor Cells

Tissue extract*	CFI activity
	% inactivation
Lung	98
Kidney	94
Liver	84
Spleen	76
Tumor extract (Novikoff)	76
Normal serum, 40–60% extract	98

* For each normal tissue (150 mg/ml) 20 μl sonicate was added to 50 μl bacterial chemotactic factor. The tumor extract was obtained from a sonicate of 10^7 tumor cells/ml. Positive and negative chemotactic counts of controls for this experiment were 315 and 15.

chemotactic activity was lost (Table VI). The data do not indicate if the tumor-derived extract has physical-chemical properties unique to that inactivator, but they do indicate the heterogenous distribution in rat tissues of inhibitors of chemotactic mediators.

As is shown in Table III, extracts from liver, spleen, and neutrophils, all of which contained a CFI activity, were found to inhibit the neutrophil chemotactic activity associated with the C3 and the C5 fragments as well as the bacterial chemotactic factor. This would suggest the wide spread presence of CFI's and the broad spectrum of activity of the CFI's.

Direct action of inactivators on chemotactic factors. Evidence was obtained to indicate that the inhibitors present in extracts isolated from Walker tumor cells, rat liver, and spleen were acting directly on the bacterial chemotactic factor. The evidence for this conclusion is shown in Tables VII and VIII. The extracts inhibited the chemotactic activity if first incubated with the bacterial factor at 37°C before heat inactivation (56°C, 1 h), whereas prior exposure of the extracts to 56°C for 1 h preceding incubation with the chemotactic factor resulted in loss of the ability to inhibit the chemotactic factor. A different approach to this question, described in Table VIII, also led to the conclusion that the extracts were blocking the chemotactic factor rather than the indicator cells (neutrophils) in the assay system. There was complete inactivation of the chemotactic activity when the extracts were incubated with the bacterial chemotactic factor, whereas addition of the extract to the cells employed in the assay system led to very little loss in chemotactic activity (Table VIII, 0–10%). The combined data in Tables VII and VIII strongly suggest that inhibition of the chemotactic system by the extracts involves a direct interaction with the chemotactic factor(s). These inhibitors seem to be related functionally to the CFI that is present in low concentrations in human serum (13).

TABLE VII

Heat Lability of CFI from Tumor Cells and Normal Rat Tissues

Bacterial factor treatment*	Chemotactic activity	Inactivation
		%
None	270	0
Walker tumor extract	15	98
Walker tumor extract heated (56°C, 1 h)	280	0
Spleen extract	85	70
Spleen extract, heated (56°C, 1 h)	275	0
Liver extract	15	95
Liver extract, heated (56°C, 1 h)	270	0

* The cell equivalent (with Walker tumor cells) was 5×10^5 cells while the tissue equivalent (with spleen and liver extracts) was 3 mg. In each case the bacterial factor and extract were incubated at 37°C for 30 min followed by 56°C for 1 h, or the extract and the bacterial chemotactic factor were separately heated at 56°C for 1 h followed by mixing and subsequent incubation at 37°C for 30 min.

TABLE VIII

Direct Inactivation of Chemotactic Factor by Extracts

Extract used as source of inactivator*	Inactivator added to: Cell suspension	Inactivator added to: Bacterial chemotactic factor
	% inactivation of chemotactic activity	
Walker tumor cells	5	100
Rat liver	0	100
Rat spleen	10	100

* Equivalent of 5×10^5 tumor cells or 3 mg tissue was incubated with 1.0 ml neutrophils (2×10^6) or with 50 μl bacterial chemotactic factor. Preparation of extracts is described elsewhere in this report. After incubation at 37°C for 30 min, treated cells or chemotactic factors were assayed in chemotactic chambers. Positive and negative chemotactic counts of control for this experiment were 300 and 40, respectively.

Ability of tumor cell CFI to inactivate monocyte chemotactic factors. As demonstrated above, CFI isolated from tumor cells will inactivate neutrophil chemotactic activity present in the fragments of C3 and C5, as well as the bacterial chemotactic factor. The same complement-dependent preparations were tested before and after incubation with the CFI-rich extract from Walker tumor cells, according to the details given in Table III. In addition, 200-μl samples of culture fluids (lymphokine) from conconavalin A-stimulated rat lymphoid cells were treated with the CFI from Walker tumor cells. The monocyte chemotactic values of the

C3, C5, and lymphocyte culture fluids were 240, 230, and 220, respectively, whereas after incubation with 50-μl samples of tumor-derived CFI, the values fell to 145, 90, and 95, respectively. The value for culture medium alone was 40. These data indicate that tumor cells contain a CFI that inhibits monocyte as well as neutrophil chemotactic factors. This is similar to the spectrum of activity for the CFI that can be isolated from human serum (13, 18, 20).

CFI activity in peritoneal fluids. Ascitic fluids from either Novikoff or Walker tumor cell-bearing rats were collected 6 days after intraperitoneal innoculation with tumor cells and tested for CFI activity. A third group of rats was injected with 20 ml 0.1% glycogen in saline and the exudates (which were rich in neutrophils) were harvested 5–6 h later. Each fluid was tested for CFI activity. The results of these studies, given in Table IX, indicate that four-fifths of ascitic fluids from Novikoff tumor-bearing rats had CFI levels that inactivated 30% or more of the chemotactic activity, that all 10 ascitic fluids from rats with Walker tumor cells demonstrated CFI activity, and that in neutrophil-rich exudates induced by glycogen the amount of CFI activity was very low, varying from 0–20%. These results suggest that tumor-associated ascitic fluids, but not acute inflammatory exudates, are rich in CFI activity.

TABLE IX

Presence of CFI in Tumor-Induced Ascitic Fluids but not in Acute Inflammatory Exudates

Cells in peritoneal cavity	Number of rats	CFI activity*	
		Individual fluids	Mean ±SEM
		% inactivation	
Novikoff tumor cells	5	30, 92, 88, 70, 0	56±32
Walker tumor cells	10	52, 46, 52, 68, 74 90, 98, 50, 67, 69	67±13
Neutrophils	7	0, 8, 12, 12, 20, 0, 10	9±5

* 50 μl of peritoneal fluids was incubated with 50 μl bacterial chemotactic factor for 30 min at 37°C followed by assay for percent inactivation of chemotactic activity. Positive and negative counts for controls in this experiment were 260 and 40, respectively.

DISCUSSION

The studies described in this report indicate that both tumor cells as well as normal rat tissues and serum contain a CFI activity that has the ability to inactivate directly and irreversibly the chemotactic activity present in a variety of leukotactic factors. What may be critical is the mechanism(s) by which such CFI's are released from tissues or leukocytes, since in most of the data presented in this report CFI was obtained only by physical disruption of cells or tissues. The finding of high levels of CFI in ascitic fluids associated with tumor cells contrasts with the low levels of CFI in exudate fluids associated with neutrophils. It is not known if the CFI in the tumor-induced ascitic fluids results from cell breakdown or is present because of some active release mechanism from tumor cells. Regardless of the explanation, it could be predicted that ascitic fluids associated with the tumor cells would have few if any leukocytes present with the tumor cells.

In a recent study it was reported that teratocarcinoma cells in mice impair the expression of the cellular inflammatory response (8). This conclusion was based on the relative lack of leukocyte infiltration into a dermal site injected with tumor cells and the finding in culture fluids (taken from tumor cells) of an inhibitor that seems to interfere with neutrophil accumulation in peritoneal cavities. Furthermore, tumor cells were found in vitro to "repel" macrophages (8). Although comparisons between our study and those of Fauve, Hevin, Jacob, Gaillard, and Jacob (8) are subject to limitation, both studies have suggested the presence of a factor that interferes with the cellular inflammatory response. Our studies provide evidence that, with at least some tumor systems, this interference is by inactivation of chemotactic mediators. The finding of CFI in tumor-induced ascitic fluids, but not in acute inflammatory cellular exudates, suggests that although many normal tissues and leukocytes contain CFI, the inactivator is released only under special circumstances. If it can be demonstrated that malignant cells, as a general rule, contain CFI and that this material is being continually released in vivo this would provide an explanation not only for the lack of a cellular inflammatory response to the malignant cells, but, if the tumor-associated CFI were to "spill over" into the circulation, this could also explain the state of "anergy" in which there would be failure to express cellular inflammatory reactions of delayed-type hypersensitivity. There is as yet no evidence presently available to indicate if nonlymphoid tumor bearing humans who are "anergic" have elevated levels of CFI in their serum. In the case of Hodgkins' disease, however, where there is good evidence that a condition of "anergy" exists, more than half the patients studied were found to have elevated levels of CFI in their serums, and, while the source of CFI is not known, it was postulated that this might represent the cause for the failure of these patients to develop cellular inflammatory reactions in the skin (20). It has been pointed out that, on the basis of the CFI findings, the "anergic" state in Hodgkins' patients may represent a defect in the effector limb of inflammation, and this abnormality would not be limited to monocytes and lymphocytes but would also involve all cells under leukotactic control (20). In the same study reports in the literature were cited to indi-

cate that in Hodgkins' disease the defect in expression of cellular inflammation is not limited to monocytes and lymphocytes, but also involves neutrophils (20). Thus, the association of CFI with malignant tumors in the rat may have local as well as systemic blocking effects on the cellular expression of the inflammatory response.

Although the mechanisms by which serum-derived CFI and tumor-associated CFI react with chemotactic factors are not known, attempts have been made to determine if the interaction between CFI and the C5 chemotactic fragments involves a binding between the two reactants. No increase was found in the sedimentation velocity of the radiolabeled C5 fragment after its inactivation by CFI, suggesting that the mechanism of inactivation does not involve a stable union between inhibitor (CFI) and chemotactic factor (18). It is known that the C3 and C5 anaphylatoxin fragments, which also possess chemotactic activity, are inactivated by the anaphylatoxin inactivator of human serum. The anaphylatoxin inactivator is a carboxypeptidase enzyme, is identical with the classical kininase of bradykinin, and inactivates both the C3 and C5 fragments as well as bradykinin by cleavage of the C-terminal arginine from each peptide (21). We have recently demonstrated that preparations of CFI inactivate bradykinin (as determined by bioassay), and that this inactivation is associated with hydrolysis of the peptide (22). In contrast to the anaphylatoxin inactivator, preparations of CFI lead to more extensive hydrolysis of bradykinin. Evidence for this conclusion comes from the release of arginine, phenylalanine, and proline (22). It is possible that the kininase activity in CFI may be due to more than one enzyme, each having similar physical-chemical parameters, but it is clear that more than a simple carboxypeptidase B-type of activity is being measured. Admittedly there is no direct evidence that the kininase activity of CFI relates to its ability to inactivate chemotactic factors, but the kininase activity, as was the case with the anaphylatoxin inactivator, may provide some clue as to the mechanism of CFI action. The solution to the problem awaits the availability of adequate amounts of highly purified chemotactic factors.

That the enzymatic activity of CFI on peptides has certain restrictions has been shown by the finding that neither angiotensin I nor angiotensin II is hydrolyzed by CFI (22). Furthermore, two CFI's can be isolated from human serum, one with a "substrate" specificity for the C3 chemotactic fragment, while the other CFI selectively inactivates the C5 chemotactic fragment. Thus, it now seems reasonable to postulate that CFI acts by enzymatic hydrolysis of chemotactic factor, and that a certain degree of substrate specificity is present.

Two papers have appeared in the literature describing the ability of soluble or particulate extracts of tumor cells to alter cell function. In the first case, a dializable factor was found in tumor fluids. This substance had the ability to inhibit adhesion of L cells as well as pseudopodia formation (23). The second paper demonstrated that extracts from tumor cells, in some cases, cause inhibition of leukocyte migration when incubated with autologous or allogeneic blood leukocytes (24). This latter finding might be related to the elaboration from lymphoid cells of a migration inhibitory factor. Thus, while both reports describe the ability of tumor cell extracts to alter functions of other cells and while such effects may be of biological significance, those reports differ significantly from the data presented in this paper in that CFI reacts with chemotactic factors and has no demonstrable direct effect on leukocytes. Thus, it must be concluded that products of tumor cells may very well affect both cellular as well as humoral factors in ways that are inimical to host defenses against tumor cells.

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