Inhibitors of Monocyte Responses to Chemotaxins Are Present in Human Cancerous Effusions and React with Monoclonal Antibodies to the P_{15}(E) Structural Protein of Retroviruses

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ABSTRACT Individuals with cancer have previously been shown to have abnormal chemotactic responsiveness. Surgical removal of the tumor often resulted in normalization of monocyte function, which suggests that human neoplasms might inhibit monocyte chemotaxis by release of soluble mediators. We therefore examined the effects of cancerous effusions on monocyte polarization, i.e., the rapid change in monocyte morphology from round to a triangular "motile" configuration in response to chemoattractants. All 17 malignant effusions, representing 15 tumor types, inhibited monocyte polarization induced by the chemoattractant *N*-formyl-methionyl-leucyl-phenylalanine by 45–89% (mean 55.9±12.7%, $P < 0.01$) in blinded assays. None of 17 benign effusions significantly inhibited polarization (0–15%, mean 6.2±4.2%). Dilutions of cancerous effusions as low as 1:200 produced inhibition that was time, temperature, and dose dependent. Monocyte polarization induced by activated serum or by chemotactic lymphokine was also blocked by cancerous effusions. The inhibitory activity affected the monocyte directly, and did not destroy the chemoattractant or block the polarization of granulocytes to chemotactic factors. High pressure liquid chromatography of five cancerous fluids revealed three peaks of inhibitory activity: ≥200,000, 46,000±13,000, and 21,000±3,000 daltons. Fractionation of noncancerous effusions revealed only small amounts of the highest molecular weight inhibitory activity. The inhibitory activity in cancerous effusion was heat stable (56°C, 30 min), trypsin sensitive, and could be absorbed by three different monoclonal antibodies reactive to P_{15}(E), a structural component of type C retroviruses. In contrast, six monoclonal antibodies with other specificities had no effect on the inhibitors of polarization. This study demonstrates that human cancerous effusions contain novel proteins that are potent inhibitors of monocyte function and that are recognized by antibodies reactive to the P_{15}(E) component of retroviruses. By producing such factors, tumor cells may subvert monocyte-mediated surveillance.

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INTRODUCTION

Macrophages, working alone or in concert with lymphocytes, can destroy cancer cells in vivo and in vitro (1, 2). Few macrophages, however, are generally found within progressively growing tumors, and their numbers are often inversely related to the tumor's metastatic potential (3–5). Moreover, cancer patients frequently have abnormally functioning monocytes, which can regain normal function after cancer therapy or tumor removal (6–10). Thus, it has been hypothesized that cancer cells may produce factors that alter monocyte-macrophage functions, thereby subverting immune surveillance (11). Indeed, in rodents, neoplasms produce agents which inhibit macrophage chemotaxis in vitro and macrophage accumulation in vivo (12–15).

A major barrier to determining the effects of neoplasms on mononuclear phagocyte function in humans

has been the lack of a suitable assay. Previous monocyte function studies, including chemotaxis (6–10), adherence (16), tumor cytotoxicity (5), and superoxide production (17), have demonstrated variable macrophage abnormalities in cancer patients, but the assays employed have been relatively cumbersome, with no sharp differentiation between cancerous and noncancerous conditions.

In this study, we utilized a new assay, quantification of monocyte polarization to chemoattractants (18), to determine whether human cancerous effusions contain factors that affect monocyte function. Polarization is defined as a morphological change in monocytes from a round to a triangular, "motile" configuration. This occurs within minutes after the exposure of the cells to chemoattractants. Effusions from patients with cancerous and noncancerous conditions were screened for their ability to affect the ability of monocytes to polarize in response to chemotactic stimuli. Inhibitors of polarization were found in all cancerous effusions studied and were characterized in terms of their biological, physical, and antigenic properties.

METHODS

Patient population. Most of the patients in this study had pleural or peritoneal effusions due to cancerous or other conditions, and they underwent paracentesis for clinically indicated diagnostic or therapeutic reasons. All cancerous effusions were cytologically proven. The patients were from the Durham Veterans Administration Hospital, the Duke University Medical Center, or North Carolina Memorial Hospital. The individuals with cancer or nonmalignant diseases (Table I) were quite similar when compared by age, sex, and nutritional status. The average serum total protein and albumin levels for the two groups were, for cancer patients, total protein, 6.0±1.0 g/dl and albumin, 3.0±0.7 g/dl; and, for noncancer patients, total protein, 6.7±0.4 g/dl and albumin 2.9±0.8 g/dl. Most patients (90%) were receiving some medications, but there were no consistent differences in those received by the two groups. The most commonly used medications were narcotics, hypnotics, vitamins, thiazides, and aminophylline. The only patients with culture-proven infection were two with empyema and one with peritonitis. These individuals as well as two others were receiving antibiotics. No patients in the cancer group had received either chemotherapy or radiation therapy for at least 2 mo before paracentesis. 8 of 22 cancer patients, however, had these treatments in the past. Most nonovarian cancer patients had metastatic diseases; 13 of 17 had metastases to several anatomic locations.

Collection of effusions. Fluids were collected in nonheparinized sterile containers and placed at 4°C or on ice. Samples were then centrifuged at 10,000 *g* for 5–10 min to remove cells and particulate matter. Effusion pH ranged from 7.2 to 7.5 and the values were unrelated to diagnosis. The pH were standardized to 7.0, the pH used for polarization and chemotaxis assays, and the fluids stored at −70°C until tested. The absorbance at 280 nm for all fluids was determined as an index of protein content.

Isolation of monocytes or granulocytes. Mononuclear leukocytes and granulocytes were isolated from the blood of normal healthy volunteers as described previously (18). Briefly, we mixed heparinized venous blood 1:1 with 3% (wt/vol) dextran (T500, Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.), allowing 20 min for sedimentation at room temperature. A mononuclear cell band was isolated from the plasma/supernate by the Ficoll-Hypaque density gradient method (19). The mononuclear cells were removed and washed with Gey's balanced salt solution con-

TABLE I
Sources of Effusions

Diagnosis	Source	Age	Sex
Cancerous Effusions			
Hepatoma	Ascites	25	M
Adenocarcinoma, pancreas	Ascites	52	M
Melanoma	Ascites	50	M
Lymphoma	Ascites	44	M
Adenocarcinoma, ovary	Ascites	21	F
Adenocarcinoma, ovary	Ascites	45	F
Adenocarcinoma, ovary	Ascites	55	F
Adenocarcinoma, ovary	Ascites	60	F
Adenocarcinoma, ovary	Ascites	69	F
Squamous, lung	Pleural	30	M
Squamous, lung	Pleural	57	M
Adenocarcinoma, lung	Pleural	53	M
Adenocarcinoma, lung	Pleural	64	M
Large cell undifferentiated, lung	Pleural	50	M
Undifferentiated, lung	Pleural	52	M
Melanosarcoma, thigh	Pleural	58	M
Liposarcoma, buttock	Pleural	71	F
Renal cell carcinoma	Pleural	35	M
Anaplastic carcinoma, unknown source	Pleural	70	M
Adenocarcinoma, breast	Breast cyst	30	F
Adenocarcinoma, colon with carcinomatous meningitis	Cerebrospinal fluid	68	F
Acute myelocytic leukemia	Pericardial	22	F
	Mean	49	
Noncancerous effusions			
Cirrhosis	Ascites	39	M
Cirrhosis	Ascites	55	M
Cirrhosis	Ascites	63	M
Cirrhosis	Ascites	65	M
Cirrhosis	Ascites	65	M
Endometriosis	Ascites	25	F
Bacterial peritonitis	Ascites	52	F
Cirrhosis	Pleural	50	M
Uremia	Pleural	64	M
Uremia	Pleural	74	M
Congestive heart failure	Pleural	62	M
Congestive heart failure	Pleural	76	M
Pulmonary embolus	Pleural	55	F
Lupus serositis	Pleural	58	F
Bacterial empyema	Pleural	56	M
Bacterial empyema	Pleural	65	M
Fibrocystic breast disease	Breast cyst	50	F
	Mean	57	

taining 2% bovine serum albumin and sodium bicarbonate (GBSS, pH 7.0, Flow Laboratories, Inc., Rockville, Md.)[1] at 4°C and 400 *g* for 15 min. After resuspension in GBSS, the cells were counted with a hemacytometer. The monocyte concentration was determined by staining for myeloperoxidase (20). The percentages of peroxidase-positive cells and monocytes as determined morphologically with a Wright-Giemsa stain were similar, ~30±10%, depending on the donor. Granulocytes were isolated from the cell pellet of the Ficoll-Hypaque density gradient as previously described (19).

Chemotactic peptides and other reagents. *N*-formyl-methionyl-leucyl-phenylalanine (FMLP) was obtained from Sigma Chemical Co., St. Louis, Mo. Zymosan-activated human serum and lymphocyte-derived chemotactic factor were prepared as previously described (21).

Polarization assay. The percentage of monocytes or granulocytes that polarized in the presence of a given stimulus was measured by incubating the cells (10^6/ml monocytes or polymorphonuclear leukocytes [PMN] in GBSS) at 37°C with the stimulus in a polypropylene tube (12 × 75 mm; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.). At times ranging from 1 to 30 min, 0.01 M ice-cold phosphate-buffered formaldehyde (10% vol/vol; pH 7.2) was added to each tube to fix the cells, in either a polarized or unpolarized state (18, 22). Cell suspensions were kept at 4°C until examined by phase-contrast microscopy. All assays were performed in duplicate and 200 cells from each tube were examined under ×400 magnification with a hemocytometer. The percentage of total cells assuming a bipolar configuration was determined.

Because the monocyte suspensions also contained lymphocytes, the percentage of polarized monocytes was determined by the formula: % monocytes polarized = (% total cells polarized/% peroxidase positive cells) × 100. Lymphocytes do not polarize in this assay (18).

Because the granulocyte suspensions were >98% granulocytes, the percentage of total cells polarized was used as the actual percentage of granulocytes polarized.

Inhibition of polarization. The ability of effusions to inhibit polarization to a chemotactic stimulus was tested by preincubating the fluids at a final concentration of 10.0, 2.0, or 0.2 absorbance 280 nm U/ml GBSS with monocytes (10^6/ml) or granulocytes (10^6/ml) for 10 min at 37°C. The chemotactic stimulant, at a concentration that induced maximum polarization, was then added to the cells for an additional 7.5 min at 37°C. Ice-cold buffered formaldehyde was then added to stop the reactions. All effusions (cancerous and noncancerous) were tested blindly and in duplicate.

Inhibition of chemotaxin-induced polarization was then calculated, after subtraction of background polarization (polarization in presence of GBSS alone), as: % inhibition = (chemotaxin polarization with buffer − chemotaxin polarization with effusion/chemotaxin polarization with buffer) × 100.

Inhibition of chemotaxis. To test fluids for ability to inhibit chemotaxis induced by FMLP, a modification of the leading front chemotaxis assay was used (23). Monocytes (1.5×10^6/ml) were preincubated with the indicated effusion at room temperature for 10 min. This mixture was then added to the top compartment of a blind-well chemotaxis chamber (21). The lower compartment contained the effusion with FMLP (10 nM). A nitrocellulose filter (5.0-μm pore size, Millipore Corp., Bedford, Mass.) separated the compartments. Chambers were incubated for 90 min at 37°C. Cancerous and noncancerous fluids were tested blindly. Counting of triplicate filters, 10 fields per filter, was done by oil immersion (×1,000). Distance (in microns) of migration into the filter by the leading front of cells (i.e., two furthest migrating cells in the same plane) was measured by the semiautomated method of Turner (24).

Effusion fractionation by high-pressure liquid chromatography (HPLC) or ultrafiltration. Cancerous and noncancerous effusions were fractionated by HPLC. Two silica-based HPLC columns were coupled in series (Micropak TSK 3000 SW, 7.5 mm × 50 cm, followed by Micropak TSK 2000 SW, 7.5 mm × 50 cm, Varian Associates, Inc., Instrument Group, Palo Alto, Calif.). Liquid phase for all chromatographic runs was 0.15 M NaCl with a flow rate of 1.5 ml/min. A 100-μl sample was fractionated, and eluted proteins were monitored by absorbance at 254 nm. Individual column fractions, kept on ice, were then assayed for stimulation or inhibition of polarization. Approximate molecular weights corresponding to the inhibitory and stimulatory fractions were determined by the method of Andrews (25).

Ultrafiltration using Amicon CF25 centriflo membrane cones (25,000 mol wt cutoff, Amicon Corp., Scientific Sys. Div., Lexington, Mass.) was also used to fractionate effusions. Effusions were standardized to 40 absorption 280 nm U/ml by dilution with phosphate-buffered saline (pH 7.0). Small portions (3–6 cm^3) were filtered through the Amicon membranes by centrifugation at 4°C and 400 *g* for 60 min. Repeat absorbance 280 measurements were done on the low-molecular weight fractions. Dilutions of 1/5, 1/100, and 1/200 were made from the low-molecular weight fractions, and these were tested for stimulation or inhibition of polarization.

Sepharose-coupled trypsin and trypsin inhibitor. Bovine pancreas trypsin and soybean trypsin inhibitor (Sigma Chemical Co.) were both coupled to cyanogen-bromide activated Sepharose 4B (Pharmacia Fine Chemicals). Material to be tested was incubated for either 30 or 60 min with the coupled Sepharose at a ratio of 0.1 ml Sepharose to 0.9 ml material. The Sepharose was removed by centrifugation.

Antibodies and other reagents. Monoclonal antibodies to murine leukemia virus $P_{15}(E)$ (both IgG_{2a} and IgG_{2b}), murine leukemia virus GP_{70} (IgG_{2a} and IgG_{2b}), and human Ia were all purchased from New England Nuclear, Boston, Mass. Three different antibodies to $P_{15}(E)$ are designated as $\alpha P_{15}(E)^a$, $\alpha P_{15}(E)^b$, and $\alpha P_{15}(E)^c$; and two different antibodies to GP_{70} are designated αGP_{70}^b and αGP_{70}^e. The isolation, characterization, and specificity of these antibodies have been previously described (26). Monoclonal antibody-producing hybrid cell lines were obtained by fusion of a BALB/c mouse myeloma line with spleen cells from 129 or C57 BL/6 mice immunized with AKR leukemia cells. The resultant cell lines produce 7S IgG antibodies. The anti-$P_{15}(E)$ antibodies react with the murine leukemia virus protein $P_{15}(E)$ or its degradation product, $P_{12}(E)$, but not with the glycoprotein, GP_{70}. The anti-GP_{70} antibodies react with GP_{70} but not with $P_{15}(E)$ or $P_{12}(E)$. Murine IgG_{2a} was kindly supplied by Dr. John Cambier of Duke University. Murine ascites fluids containing monoclonal antibodies (5–10 mg/ml) against human lymphoid cells (antibodies designated as 4F2 and 5E9) (27) and a myeloma ascites fluid of the immunoglobulin subclass IgG_1 were kindly supplied by Dr. Barton Haynes of Duke University. Formalin-fixed *Staphylococcus aureus* (IgGsorb) was obtained from The Enzyme Center, Inc., Boston, Mass. Purified murine Rauscher leukemia virus (RLV) was supplied by Dr. John Cole, Office of Program Resources and Logistics, National Cancer Institute.

[1] *Abbreviations used in this paper*: CFI, chemotactic factor inactivators; FMLP, *N*-formyl-methionyl-leucyl-phenylalanine; GBSS, Gey's balanced salt solution; HPLC, high-pressure liquid chromatography; PMN, polymorphonuclear leukocytes; RLV, Rauscher leukemia virus.

Protein determinations. Protein concentrations of ultrafiltrates were determined by the method of Lowry et al. (28).

Statistical significance. Statistical analyses were done on each daily experiment, comparing individual benign and malignant samples with FMLP controls (analysis of variance and Q-test). These data were used to compare the cumulative group of malignant fluids with the cumulative group of benign fluids (chi-square).

RESULTS

Ability of effusions to induce monocyte polarization. To determine whether effusions contained inhibitors of monocyte polarization to chemoattractants, it was first necessary to test them for their inherent ability to induce polarization. Noninfected benign and malignant fluids exhibited little intrinsic polarizing activity (Table II). These effusions also did not cause polarization at times ranging from 1 to 30 min. Ovarian cancer ascites fluids, however, when tested at the highest concentration, induced near-maximum polarization. Moreover, three of three infected benign fluids (bacterial empyema or peritonitis) induced substantial polarization. Therefore, whereas most effusions could be screened for inhibition of polarization, unfractionated ovarian cancer ascites and infected effusions could not, since the polarizing activity in the fluids would "mask" any inhibitors also present.

TABLE II
*Monocyte Polarizing Activity in Various Effusions**

Material tested	n‡	Concentration	Monocytes polarized§
			%±SE
Gey's medium alone	7		10.0±5.2
Phosphate-buffered saline alone	4		7.0±2.4
FMLP	7	10.0 nM	57.3±7.4
Zymosan-activated human serum	2	0.5%	50.0±2.8
	2	5.0%	67.0±1.4
Lymphocyte-derived chemotactic factor	2	5.0%	37.0±1.4
	2	20.0%	62.0±1.4
Benign effusions	12	10.0 abs 280 nm	17.6±8.9
	4	2.0 abs 280 nm	6.8±3.1
	4	0.2 abs 280 nm	4.5±2.4
Infected effusions	3	10.0 abs 280 nm	41.4±7.3
	3	2.0 abs 280 nm	39.0±6.1
	3	0.2 abs 280 nm	30.0±4.8
Nonovarian malignant effusions	16	10.0 abs 280 nm	19.5±6.5
	8	2.0 abs 280 nm	10.1±3.4
	7	0.2 abs 280 nm	6.9±3.1
Ovarian malignant ascites	5	10.0 abs 280 nm	55.3±3.7
	5	2.0 abs 280 nm	41.0±4.1
	5	0.2 abs 280 nm	34.8±3.7

* The inherent capabilities of the various effusions to cause monocyte polarization were compared with the capabilities of known chemotaxins.

‡ *n*, number of samples tested, each in duplicate.

§ Monocytes (10^6/ml) were incubated with the indicated material for 17.5 min at 37°C, fixed, and the percentage of polarized monocytes in duplicate tubes determined by phase-contrast microscopy (×400): % monocytes polarized = (% total cells polarized/% peroxidase positive cells in initial cell suspension) × 100.

Inhibition of polarization by malignant effusions. To test whether cancerous fluids contained inhibitors of monocyte polarization, normal monocytes were incubated with the various fluids and the cells then further incubated with FMLP at a maximum stimulatory concentration (Fig. 1). At the highest concentration tested all 16 nonovarian cancerous effusions inhibited polarization (inhibition 55.9±12.7%, $P < 0.01$), whereas none of the 17 benign fluids inhibited significantly (inhibition 6.7±4.2%) at corresponding concentrations. One cancerous effusion could not be tested at the highest concentration, because it caused clumping of cells.

In the polarization assay the extent of inhibition produced by malignant effusions was dependent on temperature and incubation time. Maximal inhibition required 30 min at 25°C but only 10 min at 37°C. Benign effusions produced insignificant inhibition, regardless of incubation time.

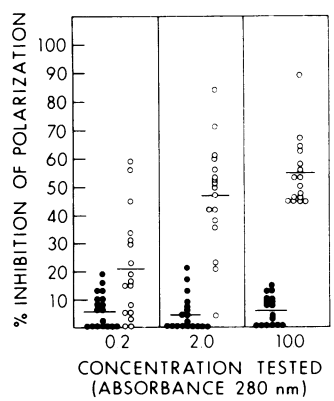

FIGURE 1 Effect of 17 cancerous (○) and 17 noncancerous (●) effusions on monocyte polarization. Monocytes (10^6/ml) were preincubated with various effusion concentrations for 10 min at 37°C, stimulated with 10 nM FMLP for 7.5 min, fixed, and the percentage of polarized monocytes determined. After subtraction of background (cells with buffer only), percentage of inhibition of polarization was calculated as: % inhibition = (FMLP polarization with buffer − FMLP polarization with effusion/FMLP polarization with buffer) × 100. At 10.0 absorbance 280 nm, all cancerous effusions, but no noncancerous effusions, caused statistically significant inhibition of FMLP-induced polarization ($P < 0.01$ by analysis of variance, Q-test, and chi^2).

To determine whether the inhibitors present in cancerous effusion could reverse polarization, normal monocytes were first stimulated with FMLP for 10 min, then cancerous effusion was added and the cells incubated for varying lengths of time before being fixed. The results of two experiments (data not shown) indicated that addition of cancerous effusion to polarized monocytes reversed that polarization to the same levels obtained by preincubation of the cells with effusion, and that this reversal was complete within 15 min after the effusion was added.

Fluids from patients with a wide variety of solid tumors contained inhibitory activity. Even chylous ascites (mixed histiocytic-lymphocytic lymphoma) and leukemic (acute myelomonocytic) pericardial effusion contained inhibitory activity. Cerebrospinal fluid from a patient with carcinomatous meningitis due to metastatic colon cancer and malignant breast cyst fluid were also inhibitory. As anticipated, unfractionated malignant ovarian ascites fluid did not appear to depress the response to FMLP, because the ascites itself induced polarization, particularly at high concentrations (Table III). At lower concentrations, however, the polarizing activity of one of the two ovarian ascites was diluted out and inhibitory activity became evident.

Inhibition of monocyte chemotaxis by malignant effusions. Several effusions were assayed for their ability to inhibit monocyte chemotaxis. The mean inhibitory activity, at each dose tested, was greater in cancerous fluids (Fig. 2). Even at the highest dose tested, however, only 11 of 17 malignant fluids were inhibitory (59.7±25.7%, $P < 0.05$). Three of nine be-

TABLE III

Effect of Ultrafiltration on Polarizing and Inhibitory Activities of Effusions

Source of effusion	Dilution tested	Ultrafiltration*	Monocyte polarization‡	Inhibition of FMLP polarization§
			%±SE	
Ovarian cancer ascites 1	1:5	—	49.7±5.0	0±9.0
	1:20	—	36.4±3.2	9.0±2.4
	1:200	—	3.2±0	33.2±14.6
	1:5	+	3.2±10.0	41.3±10.1
	1:20	+	0±3.2	85.4±18.2
	1:200	+	7.0±1.0	41.7±6.1
Ovarian cancer ascites 2	1:5	—	43.2±8.5	0±0
	1:20	—	26.4±1.8	0±0
	1:200	—	3.2±10.0	0±0
	1:5	+	0±1.6	41.3±1.9
	1:20	+	5.0±1.8	41.7±6.1
	1:200	+	0±1.7	49.5±10.2
Bacterial peritonitis ascites	1:5	—	54.0±7.0	0±0
	1:20	—	24.0±2.8	2.0±1.1
	1:200	—	18.0±2.8	0±0.6
	1:5	+	0±2.8	2.0±1.6
	1:20	+	0±4.1	2.0±0.9
	1:200	+	0±1.6	0±0.7

* The effusions were standardized to 40 absorbance 280 U/ml and individually ultrafiltered through an Amicon CF25 centriflo cone (<25,000 mol wt exclusion limit) at 400 *g* and 4°C for 60 min; + denotes ultrafiltered fraction; −, no ultrafiltration.

‡ The fluids at the indicated dilutions were incubated with monocytes (10^6/ml) for 17.5 min at 37°C, fixed, and the percentage of polarized monocytes in duplicate tubes determined by phase-contrast microscopy (×400): % monocytes polarized = (% total cells polarized/ % peroxidase positive cells (monocytes) in initial cell suspension) × 100.

§ The ability of various effusion dilutions to inhibit monocyte polarization induced by FMLP was determined by incubating cells with fluid for 10 min at 37°C, challenging with 10 nM FMLP for an additional 7.5 min at 37°C, fixing the cells, and examining them using phase contrast microscopy (×400). Inhibition of polarization to FMLP was calculated, after subtraction of background polarization (to buffer alone), as in Fig 1.

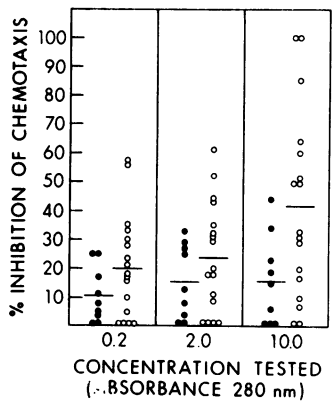

FIGURE 2 Effect of 17 cancerous (○) and 9 noncancerous (●) effusions on monocyte chemotaxis. Monocytes (1.5×10^6/ml) were preincubated with the indicated effusion concentration for 10 min at room temperature. This mixture, placed in the upper compartment of a chemotaxis chamber, was separated by a 5.0-μm pore-size nitrocellulose filter from 10 nM FMLP placed in the lower compartment in the same concentration of effusion. The chambers were incubated for 90 min at 37°C. The distance of migration of the leading front of monocytes through the stained filters was measured. After subtraction of background (migration of cells to buffer alone), percentage of inhibition of chemotaxis was calculated as: % inhibition = (FMLP chemotaxis with buffer − FMLP chemotaxis with effusion/FMLP chemotaxis with buffer) × 100. At 10.0 absorbance 280 nm, 11 of 17 cancerous and 3 of 9 noncancerous effusions caused statistically significant inhibition of chemotaxis ($P \leq 0.05$ by analysis of variance and Q-test).

nign fluids also displayed inhibitory activity (>20% inhibition). It is unlikely that chemotactic deactivation was responsible for the inhibition of chemotaxis caused by either benign or malignant effusions, because only some of the inhibitory effusions contained chemotactic activity and only at the highest concentrations tested (10 absorbance 280 nm U/ml). The presence of intrinsic chemotactic activity did not distinguish between benign and malignant effusions nor did it correlate with the degree of inhibition of chemotaxis to FMLP. In any case, the chemotaxis assay was not as effective as the polarization assay in distinguishing between cancerous and benign fluids.

Fractionation of effusions by HPLC. Several effusions were fractionated by HPLC to determine whether polarization inhibitor(s) and stimulant(s) were separable by molecular weight. This fractionation would also indicate whether any inhibitory activity in ovarian cancer ascites or infected benign effusions was masked by polarizing activity present in the unfractionated state. HPLC of malignant ovarian ascites revealed a major peak that stimulated polarization, at mol wt ≥ 200,000 (Fig. 3). A high-molecular-weight inhibitory peak was also found. The major inhibitory activity, however, was found in two peaks: one eluting just before ribonuclease, the other with ovalbumin. The approximate molecular weights of these peaks, determined by the technique of Andrews (25), were 20,000 and 45,000.

Column fractionation of malignant melanoma pleural fluid confirmed three broad but distinct inhibitory peaks (Fig. 4A). The unfractionated fluid did not polarize, and stimulatory fractions were not found (data not shown). Column fractionation of benign pulmonary infarction pleural fluid uncovered only the highest molecular weight inhibitory peak (Fig. 4B). No polarizing peaks were found. The unfractionated benign effusion neither inhibited nor stimulated polarization.

HPLC has revealed the highest molecular weight peak as well as the two low molecular weight inhibitory peaks in all five malignant effusions tested (melanoma pleural, liposarcoma pleural, lung adenocarcinoma pleural, prostate adenocarcinoma pleural, and ovarian peritoneal). Only the highest molecular weight peak (≥200,000) was found in fractions from four benign effusions (uremic pleural, pulmonary infarction pleural, heart failure pleural, and cirrhosis peritoneal) and in four sera from patients without cancer (results not shown). The approximate mean molecular weights of the three inhibitory peaks in the malignant effusions were ≥200,000; 46,000±13,000 (range 36,000–52,000); and 21,000±3,000 (range 18,000–25,000).

Since the polarizing activity of ovarian ascites was of high molecular weight, and most of the inhibitory activity of low molecular weight, Amicon cone ultrafiltration (mol wt < 25,000) was used to separate these activities. In two of two ovarian samples, ultrafiltration resulted in significant inhibitory activity and no polarizing activity in the ultrafiltrate (Table III). One infected benign effusion (bacterial peritonitis) was also ultrafiltered to see whether any low molecular weight inhibitors could be separated (Table III). The low molecular weight portion did not contain inhibitory activity.

Site of action of inhibitors of polarization. To determine whether the inhibitory activity of cancer fluids was directed at the chemoattractant or at the cell, experiments using preincubation techniques were designed. Malignant fluids were preincubated at 37°C for 10 min with either FMLP alone or the monocytes alone. After this, either FMLP or monocytes were added to the incubation mixtures for an additional 7.5 min at 37°C. The cells were then fixed, and polarization was measured. The results suggested that the inhibitory factor(s) affected the monocytes rather than FMLP (Table IV), because preincubation of fluids with cells resulted in inhibition, whereas preincubation of fluids with FMLP did not.

To test further whether the inhibitors were cell-directed, monocytes (in 5 ml) were preincubated with fluid ultrafiltrates and then washed four times with

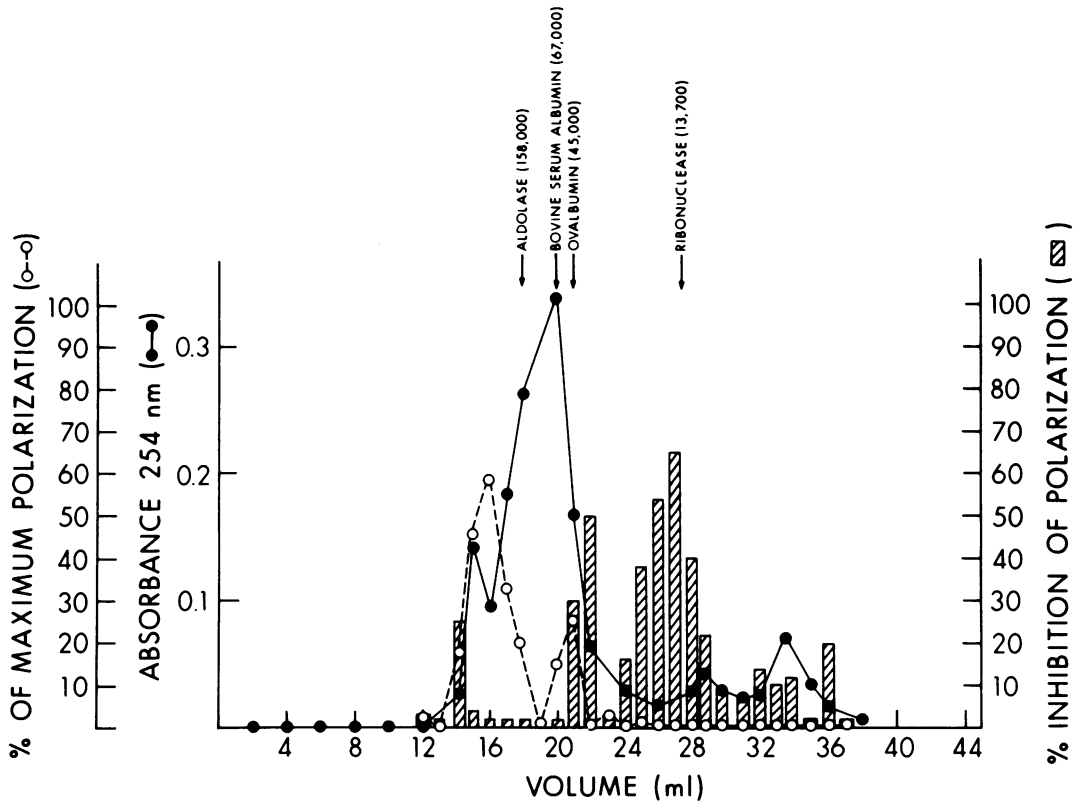

FIGURE 3 Separation by HPLC of cancerous ovarian ascites. A 100-μl sample of undiluted ascites was chromatographed on two silica-based columns coupled in series. Fractions of 1.0 ml each were monitored for absorbance at 254 nm (●). Individual fractions were assayed for stimulation (○) or inhibition (▨) of monocyte polarization.

50 ml of buffer. Upon stimulation with FMLP, monocyte polarization was still strongly inhibited in cells that had been exposed to cancerous ultrafiltrate and then washed (Table V).

Inhibition of polarization induced by several chemoattractants. To see whether malignant fluids inhibit polarization to chemoattractants other than FMLP, zymosan-activated human serum, or lymphocyte-derived chemotactic factor was used as the chemotactic factor. Polarization induced by all three agents was inhibited by malignant but not by benign effusions (Table VI).

Effects of effusions on the polarization of PMN. To test the specificity of the inhibitory activity for monocytes, malignant effusions were also incubated with PMN, and FMLP-induced polarization was measured. No inhibition of PMN polarization occurred, despite varying malignant fluid concentration, FMLP concentrations, and incubation times (Table VII).

Temperature and protease sensitivity. Ultrafiltered effusion from a patient with cancer was incubated for varying times at 37°, 56°, or 70°C and then tested for its effects on monocyte polarization. An ultrafiltrate that had been kept on ice was used as control. Incubation for 1 h at 37°C resulted in no loss of activity while 2 h resulted in a 30% decrease in activity. Activity was stable after 30 min at 56°C but was only half as potent after 60 min. Incubation at 70°C for even 10 min resulted in the loss of inhibitory activity. Ultrafiltered malignant effusion was tested for protease sensitivity by incubation with trypsin coupled to Sepharose. Incubation for 30 or 60 min at 22°C caused a decrease in the inhibition of polarization from 60% to 9 and 10%, respectively. Incubation with trypsin inhibitor coupled to Sepharose had no effect on the inhibitory activity.

Removal of inhibitory activity by monoclonal antibody. The ultrafiltered effusions (containing ~150–250 μg protein/ml) from eight different cancer patients were individually incubated with monoclonal antibody reactive to murine leukemia virus $P_{15}(E)$, or with other antibody preparations, adjusted to contain the same amount of protein as the anti-$P_{15}(E)$. The antibodies were then removed by adsorption to formalin-fixed *S. aureus*, and the materials tested for effects on monocyte polarization (Table VIII). There was a marked decrease ($P < 0.01$) in the inhibitory activity of the

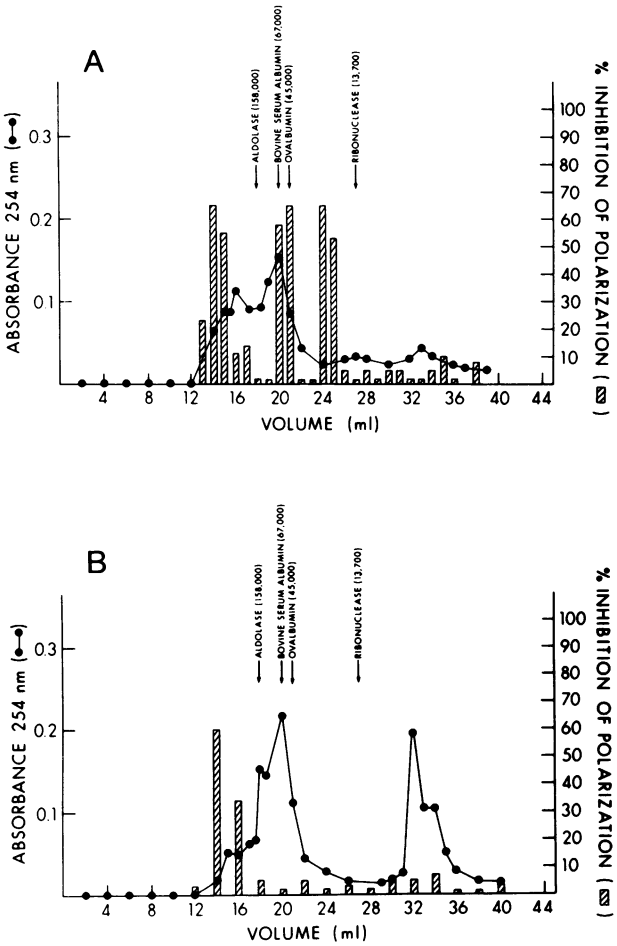

FIGURE 4 Separation by HPLC of cancerous pleural effusion due to melanoma (A) and noncancerous pleural effusion due to a pulmonary embolus (B). A 100-μl sample of undiluted effusion was chromatographed on two silica-based columns coupled in series. Fractions of 1.0 ml each were monitored by absorbance at 254 nm (●). Individual fractions were assayed for inhibition (▨) or stimulation (not plotted because they were negligible) of monocyte polarization.

filtrates absorbed with monoclonal antibody to $P_{15}(E)$. Anti-$P_{15}(E)$ antibodies of either the IgG_{2a} or IgG_{2b} subclasses were capable of absorbing the inhibitory activity even though they are reported to react with different determinants on the $P_{15}(E)$ molecule (26). There was no significant decrease in the inhibitory activity of the filtrates after absorption with mouse IgG_{2a}, monoclonal antibody to the murine leukemia virus glycoprotein GP_{70}, monoclonal antibody to human Ia antigen, a murine IgG_1 myeloma ascites fluid, or two ascites fluids containing monoclonal antibodies reactive with human lymphoid cells.

A low molecular weight (<25,000) extract of sonicated RLV, a virus known to contain $P_{15}(E)$, was also tested for inhibition of the polarization of human monocytes (Table VIII). The extract significantly inhibited monocyte polarization and the inhibitory activity was absorbed by monoclonal anti-$P_{15}(E)$ antibodies of both the IgG_{2a} and IgG_{2b} subclasses. Murine IgG_{2a} and monoclonal anti-GP_{70} had no effect on this inhibitory activity.

TABLE IV
Monocyte Polarization After Preincubation of Cells or Chemoattractant with Cancerous Ascites

Material preincubated with ascites*	Ascites concentration	Inhibition of polarization‡ Experiment 1	Experiment 2
	abs 280 nm	*%±SE*	
Monocytes	10.0	67.6±2.8	67.0±4.2
	2.0	51.1±1.4	—§
	0.2	46.6±1.4	22.0±2.8
Chemoattractant (FMLP)	10.0	0±0	0±4.6
	2.0	0±3.1	—§
	0.2	0±0	9.1±1.4

* Ascites at the indicated concentrations was preincubated in duplicate tubes with either monocytes or chemoattractant (10 nM FMLP) for 10 min at 37°C. Either monocytes or chemoattractant were added for an additional 7.5 min at 37°C, the cells fixed and examined by phase-contrast microscopy (×400).
‡ Inhibition of polarization to FMLP was calculated, after subtraction of background polarization (to buffer alone), as in Fig. 1.
§ Not determined.

TABLE V
Monocyte Polarization after Washing of Cells Preincubated with Ultrafiltrates of Effusions

Source of effusion*	Inhibition of polarization‡ Unwashed monocytes	Washed monocytes
	%±SE	
Squamous carcinoma pleural effusion	55.5±4.0	48.3±3.1
Lymphoma pleural effusion	70.4±4.0	61.0±8.5
Cirrhosis ascites	5.5±2.1	5.5±3.8
Congestive heart failure pleural effusion	0.0±1.2	7.0±4.5

* Ultrafiltrates of effusions (diluted 1:5) were preincubated with normal monocytes for 10 min at 37°C. The cells (5 ml) were then washed four times in buffer (50 ml), stimulated with chemoattractant (10 nM FMLP) for 7.5 min at 37°C, fixed, and then examined by phase-contrast microscopy (×400).
‡ Inhibition of polarization to FMLP was calculated, after subtraction of background polarization (to buffer alone), as in Fig. 1.

TABLE VI

*Effect of Effusions on Monocyte Polarization Induced by Various Chemoattractants**

Source of effusions	Concentration	% Inhibition of polarization‡		
		FMLP	Zymosan-activated human serum	Lymphocyte-derived chemotactic factor
	absorbance 280 nm	*10 nM*	*5% vol/vol*	*20% vol/vol*
Melanoma ascites	10.0	98.0±1.4	71.8±13.1	83.0±9.8
	2.0	77.0±2.8	67.5±19.1	62.2±1.4
	0.2	0.0±10.6	20.2±6.1	21.5±3.2
Melanosarcoma pleural effusion	10.0	81.2±9.8	75.6±12.1	100.0±0.0
	2.0	62.0±0.7	67.7±9.8	55.4±4.5
	0.2	8.0±0.0	0.3±0.7	10.2±2.1
Squamous carcinoma pleural effusion	10.0	91.6±12.7	77.7±14.0	97.7±3.5
	2.0	46.1±11.3	8.6±3.2	86.7±9.8
	0.2	7.7±6.8	8.6±3.2	52.7±4.5
Cirrhosis ascites	10.0	0±1.4	0±0	0±1.3
	2.0	0±0	0±0	20.5±9.7
	0.2	0±0	0±3.2	0±1.3
Congestive heart failure pleural effusion	10.0	0±1.8	0±0	10.0±2.4
	2.0	0±0	0±2.8	0±0
	0.2	0±0	0±0	0±1.3

* Various cancerous or noncancerous effusions were preincubated with monocytes (10^6/ml) for 10 min at 37°C, the cells were then challenged with the indicated chemoattractant for an additional 7.5 min at 37°C, fixed, and examined by phase-contrast microscopy (×400).

‡ Percent inhibition of polarization to the indicated chemoattractant (Ctx) was calculated, after subtraction of background polarization (to buffer alone), as: % inhibition = (Ctx polarization with buffer − Ctx polarization with effusion/Ctx polarization with buffer) × 100.

Four malignant effusions that had not been ultrafiltered were also tested before and after absorption with anti-P_{15}(E) (Table IX). The monoclonal anti-P_{15}(E) removed a significant portion of the activity from these fluids. Murine IgG_{2a} or monoclonal anti-GP_{70} had no significant effect on inhibition.

To see whether the inhibitors which affected polarization and chemotaxis were antigenically related, five effusions which inhibited chemotaxis were preincubated with buffer alone or with buffer containing either monoclonal anti-P_{15}(E) or monoclonal anti-GP_{70}. Antibody was removed by *S. aureus* absorption and the fluids were tested for their ability to inhibit chemotaxis. The average inhibition of the five effusions after preincubation with buffer or anti-GP_{70} was 72 and 77%, respectively, whereas the fluids absorbed with anti-P_{15}(E) inhibited chemotaxis by an average of only 33% (data not shown).

In the absence of FMLP, effusions or ultrafiltrates treated with anti-P_{15}(E) plus *S. aureus* had no polarizing or chemotactic activity. Thus, the ability of anti-P_{15}(E) to block the inhibitory activity in malignant effusions was not due to the generation of polarizing or chemotactic activity.

DISCUSSION

Although the concept of immune surveillance, as a mechanism of host resistance to cancer, offers hope that highly specific, more effective, and less toxic therapies for tumors can be developed, extensive clinical immunotherapy trials (29, 30) have not yet fulfilled expectations. In fact, the role of the immune system in cancer control has been seriously questioned. Many arguments against immune surveillance, however, underplay the role of the macrophage. Evidence supporting the importance of the macrophage in resisting the development and spread of cancer has recently received increasing attention (11).

Since abnormalities of monocyte activities, particularly chemotaxis, have been reported in humans and animals with cancer (6–17), the present study sought to determine whether inhibitors of monocyte function were present in human cancerous effusions. A new assay, monocyte polarization in response to chemoattractants, proved to be rapid and reproducible in blind experiments. More importantly, the polarization assay appears to be sensitive and specific in detecting monocyte inhibitors in the effusions of cancer

TABLE VII
*Effect of Cancerous Effusion on the Polarization of PMN**

Effusion concentration	Concentration of FMLP	Inhibition of polarization‡		
		Monocytes	PMN	
		10-min preincubation	10-min preincubation	20-min preincubation
absorbance 280 nm	*nM*		%	
10.0	10.0	44.2±4.6	0.0	13.6±7.1
2.0	10.0	42.1±5.1	0±0.8	0.5±0.5
0.2	10.0	—§	0±0	4.2±2.1
10.0	1.0	—	0±0	—
2.0	1.0	—	3.1±1.4	—
0.2	1.0	—	0±0	—
10.0	0.1	—	0±0	0±0.9
2.0	0.1	—	0±0	0±2.1
10.0	0.01	—	0±0	—
2.0	0.01	—	2.2±1.4	—

* Cancerous effusion was preincubated with normal granulocytes (PMN, 10^6/ml) or monocytes (10^6/ml) for 10 or 20 min at 37°C, the cells challenged with FMLP at the indicated concentrations for 7.5 min at 37°C, fixed and then examined by phase-contrast microscopy (×400).

‡ Percentage of inhibition of polarization to FMLP was calculated, after subtraction of background polarization (to buffer alone), as in Fig. 1.

§ Not determined.

patients. All 17 nonovarian cancerous effusions tested, but none of 17 noncancerous fluids tested, contained activity that inhibited polarization. Since ovarian cancer ascites contained high molecular weight intrinsic polarizing activity, inhibitors could only be found in the ovarian fluid ultrafiltrates or low molecular weight fractions separated by HPLC. The finding that ovarian ascites fluids contained polarizing as well as inhibitory activities suggests that, at least with some neoplasms, a complex interplay between stimulatory and suppressive mechanisms may be operative. The balance between these activities, particularly within the tumor itself, could be one determinant of the growth potential of the neoplasm.

A striking finding was the association of these inhibitors with 15 varied solid tumors of several cell types (squamous, adenocarcinoma, undifferentiated, sarcoma, melanoma, etc.) metastatic from numerous primary anatomic locations. Even focal exudates in a patient with leukemia and another with lymphoma contained inhibitors. One malignant cerebrospinal fluid sample also contained inhibitory activity, which suggests that inhibitors can be detected in fluids from a variety of locations. Variables such as sex, age, medications, and nutritional status did not correlate with inhibitory activity. Chemotaxis experiments revealed similar inhibitory activity in cancer effusions, but the polarization assay is more suitable for detecting inhibitors of monocyte function.

In characterizing the inhibitory activity by means of high pressure gel filtration, three fractions of ~ ≥200,000, 46,000±13,000 and 21,000±3,000 daltons were found. Only the highest molecular weight component was present in noncancerous effusions or in normal human serum.

The inhibitory activity found in human cancerous effusions was biologically potent, being active at dilutions of up to 1:200, and appeared to at least partially reside in a protein moiety, because the activity of low molecular weight ultrafiltrates was destroyed by trypsin. This is contrary to the lipid-like inhibitory factor isolated from cell cultures of human breast cancer or melanoma (31). The inhibitory factors for monocyte polarization are cell-directed and monocyte-specific, in that granulocyte polarization was unaffected. This is similar to the inhibitors of macrophage chemotaxis produced by murine neoplasms (12). The human inhibitors, moreover, blocked monocyte polarization to all three classes of chemoattractants tested.

Inhibitors of monocyte and granulocyte function had been previously described in normal serum (32, 33). Most of the previously described inhibitors have been

chemotactic factor inactivators (CFI) although cell-directed inhibitors were also reported. Increased levels of CFI or cell-directed inhibitors have been found, however, in sera from patients with noncancerous diseases such as cirrhosis, sarcoidosis, leprosy, systemic lupus erythematosus, and chronic periodontitis. Occasional malignant states, especially Hodgkin's disease, have been associated with increased serum levels of CFI or cell-directed inhibitors. Kjeldsberg and Pay (34) described CFI in sera in 19 of 21 patients with cancer of the lung or prostate. No CFI was found in 12 controls. Monocyte chemotaxis was also abnormal in 10 of the cancer patients. In four patients treated and followed, two treated surgically lost CFI, two treated chemotherapeutically did not lose CFI. These CFI and cell-directed inhibitors were described as larger proteins (4, 7, and 10S sedimentation coefficients) than those inhibitors described in this paper.

In previous studies we found that potent inhibition of macrophage accumulation in mice was caused by the injection of a murine leukemia virus envelope protein of 15,000 daltons, termed $P_{15}(E)$ (35). The inhibitory activities for macrophage accumulation derived from either murine tumors or disrupted murine leukemia viruses were physiochemically and antigenically similar. Antibody raised against disrupted Friend leukemia virus absorbed the inhibitory activity for macrophage accumulation isolated from a murine tumor cell extract (36). Recent studies by other laboratories have also demonstrated the immunosuppressive potential of type C virus structural proteins. Mathes et al. (37, 38) showed that a 15,000 mol wt protein of feline leukemia virus could abrogate lymphocyte blastogenesis and capping in vitro and reduce the level of antibody formation in vivo. Inhibition of in vitro lymhocyte transformation by disrupted murine leukemia virus has been demonstrated by Fowler et al. (39).

In the present study we have demonstrated that the low molecular weight inhibitors of monocyte polarization in human effusions are similar to the murine inhibitor of macrophage chemotaxis derived from neoplasms (12). More importantly, a monoclonal antibody reactive against the $P_{15}(E)$ component of murine leukemia viruses bound the inhibitory activity derived from all human cancerous effusions tested. These data suggest that the human inhibitory factor may have antigens in common with a structural component of a murine RNA tumor virus. In addition, extracts of a murine leukemia virus also inhibited human monocyte polarization and this activity was also absorbed by antibody to $P_{15}(E)$. We can not yet estimate, however, the total extent of the antigenic or structural relationship be-

TABLE VIII

*Effect of Various Monoclonal Antibodies on Inhibition of Monocyte Polarization by Ultrafiltrates of Cancerous Effusions**

Ultrafiltrate preincubated with‡	% Inhibition (±SE) of monocyte polarization by ultrafiltrate from effusion§											
	A‖	A	B‖	C‖	D	D	E	F	G	H	RLV	RLV
Media alone	60.8±16.6	63.6±3.2	55.0±1.4	67.6±1.1	62.2±7.2	60.0±4.8	61.1±11.0	46.5±8.6	58.4±4.3	70.9±3.0	66.7±15.5	52.5±3.2
$\alpha P_{15}(E)^a$ (IgG_{2a})	16.0±0	0±16.5	12.6±3.2	9.4±0	—¶	0±2.8	0±7.8	7.9±1.5	4.3±1.2	—	3.1±1.4	0±7.7
$\alpha P_{15}(E)^b$ (IgG_{2b})	—	1.0±10.9	—	—	—	—	—	—	—	—	—	2.0±0.7
$\alpha P_{15}(E)^c$ (IgG_{2b})	—	11.2±4.6	—	—	18.4±0	—	—	—	—	15.4±10.2	—	8.4±7.2
$\alpha GP_{70}{}^b$ (IgG_{2a})	63.2±14.2	46.4±1.5	66.6±9.5	63.0±12.3	—	—	—	52.4±9.0	50.0±0	—	—	50.5±2.7
$\alpha GP_{70}{}^c$ (IgG_{2b})	—	52.2±2.7	—	—	—	—	—	—	—	—	—	53.0±9.2
IgG_{2a}	67.5±18.3	—	55.0±10.6	63.0±0	—	59.5±1.5	54.5±1.2	57.9±9.0	56.7±12.0	—	65.8±0.9	—
αIa	61.±12.3	—	61.2±11.6	69.8±12.3	—	—	—	—	—	—	—	—
Myeloma IgG_1	57.2±4.3	—	55.0±8.5	72.0±1.1	54.7±7.2	—	—	—	—	56.2±8.7	—	—
4F2	49.8±3.1	—	59.1±9.6	61.5±3.9	—	—	—	—	—	—	—	—
5E9	66.3±13.5	—	50.8±2.0	62.6±8.3	—	—	—	—	—	—	—	—

* Ultrafiltrates were prepared from the following effusions by centrifugation through Amicon CF25 centriflo cones: (A) ascites fluid, ovarian carcinoma; (B) ascites fluid, ovarian carcinoma; (C) ascites fluid, melanoma; (D) pleural fluid, melanoma; (E) pleural fluid, metastatic squamous cell carcinoma of the lung; (F) pleural fluid, metastatic anaplastic carcinoma of unknown origin; (G) pleural fluid, squamous cell carcinoma of the lung; (H) ascites fluid, lymphoma; (RLV) murine Rauscher leukemia virus.

‡ 0.5 ml of the ultrafiltrate was incubated for 15 min at 22°C with 10 μl of GBSS or GBSS containing 7.5 μg of the appropriate antibody. 25 μl of formalin-fixed *S. aureus* (IgGsorb) was added, the mixture incubated an additional 12 min at 22°C, and the IgGsorb removed by centrifugation at 12,000 *g* for 2 min.

§ 0.1 ml of absorbed ultrafiltrate was incubated for 10 min at 37°C with 0.3 ml of cell suspension containing 4.8×10^5 peroxidase-positive mononuclear cells. 0.1 ml of GBSS or 50 nM FMLP in GBSS was then added to each of duplicate tubes, the tubes incubated an additional 7.5 min at 37°C, the cells fixed, and the percentage of polarized monocytes determined. The percentage of inhibition of polarization to FMLP was calculated, after subtraction of background polarization (to buffer alone), as in Fig. 1.

‖ These ultrafiltrates were each absorbed with 2.5 μg of the appropriate antibody.

¶ Not determined.

TABLE IX

Effect of Monoclonal Antibodies on Inhibition of Monocyte Polarization by Unfractionated Cancerous Effusions

Experiment	Effusion*	Inhibition of monocyte polarization after preincubation with‡			
		GBSS	$\alpha P_{15}(E)^a$ (IgG_{2a})	IgG_{2a}	$\alpha GP_{70}{}^b$ (IgG_{2a})
		% ±SE			
1	A	54.3±4.3	0±9.8	57.6±17.9	58.4±13.0
	B	47.4±14.2	0±6.6	51.3±5.5	50.4±3.0
	C	64.2±1.6	0.7±13.9	49.8±6.3	52.6±3.3
2§	A	53.9±3.3	11.6±11.6	—‖	—
	C	84.5±7.7	17.4±24.8	—	—
	D	72.9±0	30.6±12.9	—	78.4±15.5
3§	A	66.3±7.8	6.7±2.6	—	—
	C	43.0±10.3	19.6±2.7	—	—
	D	81.9±5.3	31.5±2.6	—	70.2±9.1

* Effusions tested were as follows: (A) pleural fluid, squamous cell carcinoma of the lung; (B) pleural fluid, melanoma; (C) ascites fluid melanoma; (D) pleural fluid, carcinoma of the bladder.

‡ 0.5 ml of effusion was incubated for 15 min at 22°C with 10 μl of GBSS or GBSS containing 7.5 μg of the appropriate antibody. 25 μl of IgGsorb was added, the mixture incubated an additional 12 min at 22°C, and the IgGsorb removed by centrifugation at 12,000 *g* for 2 min. 0.10 ml of the absorbed effusion was then incubated for 10 min at 37°C with 0.3 ml of cell suspension containing 4.8×10^5 peroxidase-positive mononuclear cells. 0.10 ml of GBSS or 50 nM FMLP was then added to each of duplicate tubes, the tubes incubated an additional 7.5 min at 37°C, the cells fixed and the percentage of polarized cells determined. The percent inhibition of polarization to FMLP was calculated, after subtraction of background polarization (to buffer alone), as in Fig. 1.

§ In these experiments 15.0 μg of the indicated antibodies were used.

‖ Not determined.

tween the human and murine material. A majority of the inhibitory activity in unfractionated human cancerous effusions was absorbed by monoclonal anti-$P_{15}(E)$. This suggests that the high molecular weight inhibitory activity detected by gel filtration chromatography of cancer effusions may be either precursor molecules or aggregates of the low molecular weight inhibitor.

The significance of identifying a protein in human cancerous effusions bound by antibody reactive with murine leukemia virus $P_{15}(E)$ is not yet clear. Our data do not provide evidence that the inhibitory factor in cancerous effusions is a result of viral infection. The inhibitory protein could be synthesized upon the derepression of genes in malignant cells that code for a virally related protein. Alternatively, the protein may be one produced as part of the membranes of de-differentiated cells, whether or not the dedifferentiation was due to virus infection. $P_{15}(E)$ is an envelope protein of type C retroviruses and thus is derived from the host cell's membrane as the virus buds from the infected cell. Thus the dedifferentiating effect of a virus infection could stimulate the host cell to produce a naturally occurring membrane protein that becomes incorporated into the viral envelope. Indeed, there is evidence for wide antigen cross-reactivity of $P_{15}(E)$ isolated from viruses of many animal species including primate. Thiel et al. (40) have recently demonstrated that rabbit antiserum against Friend murine leukemia virus $P_{15}(E)$ was capable of precipitating material from both primate type C and type D viruses.

Thus, the evidence presented here demonstrates that potent inhibitory agents that alter monocyte responsiveness to chemoattractants are present in human cancerous effusions. We have not yet determined whether these inhibitors are present systemically or are localized to focal tumor microenvironments, although studies to answer this question are ongoing. Similarly, a possible correlation between the amount of inhibitory activity with the extent of cancer metastasis or tumor burden is being investigated. In any case, assaying for inhibition of monocyte polarization to chemotactic factors provides an apparently useful assay for dif-

ferentiating cancerous from noncancerous effusions. This assay could therefore have considerable diagnostic importance. These studies, moreover, support the concept that clinically apparent neoplasms may produce potent inhibitors of monocyte function and thereby subvert their destruction by immunological processes requiring macrophage accumulation. If this is the case, immunotherapy specifically designed to counter the inhibition of monocyte response to chemoattractants may be more effective than those regimens currently being used.

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