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Inhibition of macrophage chemotaxis by supernatant fluid from malignant cell lines

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Summary

The chemotactic locomotion of peritoneal macrophages *in vitro* was measured in the presence of supernatant fluid from 27 different tumour cell lines. All the tumour-derived cells tested caused profound depression of macrophage chemotaxis towards the chemo-attractant casein. Conversely, supernates from rapidly dividing non-malignant cells uniformly failed to depress macrophage migration. This confirms the results of a previous investigation and provides further evidence that malignant tumours depress the functions of the reticuloendothelial system of the host animal *in vivo*.

Résumé

Le mouvement chimiotactique des macrophages péritoneaux *in vitro* a été mesuré en présence des surnageants provenant de vingt-sept coupes de différentes cellules de tumeurs. Toutes les cellules provenant de tumeurs testés ont causé un profond abaissement de la chimiotaxie macrophage vers l'agent chimique — la caséine. Au contraire les surnageants provenant de cellules bénignes à éclatement rapide n'ont pas réussi uniformement à empêcher la migration des macrophages. Ce fait confirme les résultats d'une recherche précédente et montre avec plus d'évidences que les tumeurs malignes entravent les fonctions du système réticulo endothélial de l'hôte (l'animal) *in vivo*.

Introduction

Previous studies [1] have clearly demonstrated that malignant tumours depress the functions of the reticuloendothelial system of the host animal. The functions investigated were intravascular colloid clearance *in vivo*, the chemotactic locomotion of macrophages *in vitro*, and the formation of macrophage colonies by bone marrow stem cells *in vitro*. Serum from tumour-bearing animals or supernatant fluid from tumour cell cultures caused profound depression of all three functions. The present investigation has tested supernates from malignant tumours of animals and man, and other rapidly dividing cells that possess no malignant properties, for their ability to depress macrophage chemotaxis towards casein.

Patients and methods

Human tumour cell lines

The human tumour cell lines used in this study were obtained from the Department of Cell Biology, University of Glasgow, U.K., and were of two types: B cell-derived (seven in number) and T cell-derived (two in number).

Human lymphoblastoid cells Bri-8, Bec 11, 1788 and Tay were Epstein–Barr (EB) virus induced from healthy adults. The cell lines Bri 8, Tay and 1788 secrete IgM; Tay and 1788 secrete large amounts while Bri 8 secretes small amounts. Bec 11 is IgG secreting. These cells clump in suspension tissue culture and grow at a concentration of between 2×10^5/ml and 2×10^6/ml, with an approximate doubling time of 24 h. Morphologically the cells have moderate amounts of rough endoplasmic reticulum and exhibit microvillus surfaces. The three remaining B cell lines Daudi, Raji and 8A are Burkitt (African childhood) lymphoma-derived and EB virus positive. Daudi and Raji carry surface IgM, high and low levels, respectively; 8A is hybrid, arising out of Daudi–Raji fusion and exhibiting surface IgM in moderate amounts.

These cells (i.e. Daudi and Raji) are morphologically lymphoblasts and grow in suspension culture without any clumping tendency. They grow to a higher density than cells of the lymphoblastoid type and are less sensitive to low density than the latter. They have smooth outer membranes, show little or no rough endoplasmic reticulum and stay at a stationary phase (high density) for prolonged periods (5–7 days). The doubling time for these cells is approximately 16 h. The T cells tested were CEM and 48.

Porcine cell line

The supernatant fluid from cultures of the endothelium of pig aorta was kindly supplied by the Beatson Institute, Glasgow, U.K.

Mouse myeloma cells

Murine myeloma cells (17 in number) were kindly provided by the Institute of Biochemistry, University of Glasgow, U.K. The cells have a generation time that ranges from 12 to 16 h, in the case of MPC 11, to 24 h in hybrid cells and 5781.4. All are cloned cell lines, except 5563T and MOPC 70A. They grow well at a density of 5×10^4/ml – 5×10^5/ml. Some cells (5563.2, 368.2 5563T, P1-1, P1-2, P1-4, MPC 11.20 and MPC 11-2) produce IgG; others (MOPC 315-40, MOPC 315-34 and MOPC 315.43) produce IgA. H5, H17 and H18 are hybrid cells.

Rat and hamster cells

Rat embryo fibroblasts (REF), baby hamster kidney (BHK) and baby hamster kidney polyoma virus-transformed (BHKP) cells were kindly supplied by the Department of Cell Biology, University of Glasgow, U.K.

Supernatant fluid from cell cultures

Cell culture supernatant fluids were prepared by filtering media through 0.22 μm millipore filters (Millipore, Bedford, U.K.).

Serum from 'tumour-bearing animals'

Blood was collected from the retro-orbital plexus by means of specially made pipettes [2], pooled and centrifuged at 1000 r.p.m. for 10 min, and serum collected. Sera were stored at −20°C until required.

In order to prepare 'serum from tumour-bearing mice', blood was obtained on days 1, 2 and 3 after a tumour (Lewis lung tumour) had been grafted to healthy mouse recipients. A mixture of equal volumes of these three blood samples was prepared and the resulting serum preserved at −20°C until required.

Macrophage suspension for chemotaxis

Mouse (CBA) peritoneal macrophages were obtained as previously described [1]. The peritoneal cavity of unstimulated mice was washed out with Gey's solution (5 ml/mouse). The washings were suspended in fresh Gey's solution and the concentration adjusted to 10^6/ml.

Macrophage chemotaxis tests

A modification of the double chamber method of Boyden was used. This technique has been described in detail previously [3].

Negative control chambers contained Gey's solution only, and positive controls contained casein (Merck, Darmstadt, FRG) 1 mg/ml in the lower chamber. Duplicate chambers were set up in all experiments. Millipore filters (8 μm pore size: Millipore, Bedford, U.K.) were used, the incubation time being 135 min. Cell migration was measured by estimating the distance in micrometres migrated by the leading front of cells by the method of Zigmond and Hirsch [4]. The results are expressed as the means of 10 readings per test. Differences in migration were considered significant only if the mean migration of the cells towards the test substance (the chemo-attractant) differed from that towards the control by ≥10 μm. It was earlier found that in tests demonstrating depression of migration, even greater depression could be obtained by pre-incubating cells with the inhibiting substance (serum or supernatant fluid) for 30 min before testing (unpublished results).

Results

Effect of serum from tumour-bearing mice on the chemotactic locomotion of macrophages towards casein

Peritoneal macrophages, at a concentration of 10^6 cells/ml of suspension, were incubated with 0.1 ml of pooled serum from C57BL mice bearing Lewis lung carcinoma. The migration of the cells in the upper chamber towards the chemotactic factor, casein, in the lower chamber was then measured. The results summarized in Table 1 clearly show that serum from tumour-bearing mice caused a marked depression in the locomotion of macrophages (60.2% and 75.2% decrease in locomotion towards 250 μg and 500 μg of casein, respectively; $P < 0.001$. Student's *t*-test). This confirms previous observations [1,5].

Effect of supernatant fluid from murine myeloma cell lines on the chemotactic locomotion of macrophages

Supernates from cultures of S91 non-metastasizing melanoma of DBA mice also depressed macrophage chemotaxis in similar tests (unpublished results). It was considered of

Table 1. Effect of murine myeloma cell lines and pig aorta endothelium cell line on macrophage locomotion

	Distance in micrometres		% decrease in locomotion	
Chemotactic factor	(250 μg)*	(500 μg)*	(250 μg)*	(500 μg)*
Serum from tumour-bearing animals	33.5 ± 0.6	25.0 ± 0.2	62.2	45.2
40	57.4 ± 0.3	58.4 ± 0.6	36.7	42.6
11.2	40.6 ± 0.7	29.4 ± 0.5	54.4	71.3
11.20	40.0 ± 0.2	41.0 ± 0.2	55.6	59.4
43	36.8 ± 0.5	29.2 ± 0.2	58.9	71.3
5781.4	31.2 ± 0.3	29.7 ± 0.1	65.6	70.3
MOPC 70A	40.1 ± 0.8	39.7 ± 0.6	55.6	60.4
P1.4	30.1 ± 0.3	36.3 ± 0.8	66.7	64.4
P1.17	35.0 ± 0.2	41.8 ± 0.2	61.1	58.4
H.5	39.8 ± 0.3	34.0 ± 0.5	55.6	66.3
5563T	24.1 ± 0.5	27.6 ± 0.3	73.3	71.3
368.2	39.9 ± 0.1	45.1 ± 0.2	55.6	55.4
5563.2	34.2 ± 0.1	33.1 ± 0.2	62.2	67.3
P.1.1	33.8 ± 0.5	32.9 ± 0.3	62.2	67.3
34	29.5 ± 0.3	32.6 ± 0.5	66.7	67.3
H 17	38.9 ± 0.2	30.8 ± 0.1	56.7	69.3
H 18	29.9 ± 0.1	29.7 ± 0.1	66.7	70.3
P.1.2	26.0 ± 0.4	39.7 ± 0.2	71.1	60.4
PAE	94.5 ± 0.3	97.8 ± 0.5	—	—

Migration in micrometres is expressed as the mean of 10 readings ± standard error of means.

Per cent decrease in locomotion = $\frac{\text{positive control} - \text{observed migration}}{\text{positive control}} \times 100.$

Negative control (Gey's solution only) = 30.1 ± 0.1.

Positive control (casein only) = 89.9 ± 0.1 and 100.5 ± 0.1 at 250 μg and 500 μg, respectively.

PAE = Pig aorta endothelium.

*Concentration of the chemo-attractant casein.

interest to test supernatant fluids from other tumours of mice for their ability to depress macrophage locomotion. Selected mouse tumour cell lines (17 myeloma cell lines) were tested. The migration of macrophages pretreated with 0.1/ml supernatant fluid was compared with the migration of a comparable number of untreated cells towards the same chemotactic stimulus. As shown in Table 1, supernatant fluid from all 17 myeloma cell lines caused a marked depression of macrophage chemotaxis. Furthermore, the tumours exhibited a wide variation in their ability to depress macrophage migration towards casein. The greater depression of macrophage locomotion was caused by the cell line 5563T (about 75% at 250 µg of casein) and the lowest by the cell line designated 40 (nearly 40%).

Effect of supernatant fluid from various human cell lines on macrophage migration

The above results clearly show that supernant fluids from cultures of various tumour cell lines of mouse origin can produce a significant depression of macrophage migration. The effect of supernatant fluid from cell lines derived from man was explored next. The cell lines tested were both B-cell lines (Daudi, Tay, BRI. 8, 1788, 8A, RAJI and BEC 11) and T-cell lines (CEM). Some (Tay, BEC 11, Bri 8 1788) were either EB virus induced from material obtained from apparently healthy individuals or were EB virus positive lines (Daudi, Raji and 8A), which were Burkitts lymphoma derived. The results (Table 2) clearly show that all the human cell lines caused a depression of macrophage chemotaxis towards casein. The highest activity was given by Daudi (77%) and the lowest by CEM (37%) at 250 µg casein; similar results were obtained for tests carried out at a higher casein concentration of 500 µg (80% and 56%, respectively).

Effects of supernatant fluid from rapidly dividing non-malignant cell lines on macrophage chemotaxis

Supernatant fluid from rapidly dividing but non-malignant cell lines, rat embryo fibroblast, baby hamster kidney and pig aorta endothelia was tested. As shown in Table 2, the supernatant fluid from these rapidly dividing cells had no effect on macrophage locomotion. Fur-

Table 2. Effect of human malignant cell lines on macrophage locomotion

Chemotactic factor	Distance in micrometres		% decrease in locomotion	
	250 µg	500 µg	250 µg	500 µg
BRI.8	21.1 ± 0.4	20.2 ± 0.2	76.7	80.2
Dandi	29.6 ± 0.2	30.8 ± 0.1	66.7	69.3
Tay	32.8 ± 0.3	30.0 ± 0.2	63.3	70.3
1788	30.2 ± 0.3	30.2 ± 0.5	66.7	68.3
8A	30.8 ± 0.2	31.0 ± 0.9	65.6	69.3
Raji	28.2 ± 0.1	28.6 ± 0.2	69.9	71.3
Bec.11	28.7 ± 0.2	28.8 ± 0.1	67.8	71.3
CEM	43.6 ± 0.4	44.2 ± 0.2	51.1	56.4

Migration in micrometres is expressed as the mean of 10 readings ± standard error.

Per cent decrease in locomotion = $\frac{\text{positive control} - \text{observed migration}}{\text{positive control}} \times 100.$

Negative control (Gey's solution only) = 30.1 ± 0.1.

Positive control (casein only) = 89.9 ± 0.1 and 100.5 ± 0.1 at 250 µg and 500 µg, respectively.

thermore, supernatant fluid derived from baby hamster kidney polyoma virus-transformed cells caused a marked depression of macrophage chemotaxis towards casein (Table 3), showing that the ability to depress macrophage function is associated with malignant transformation.

Discussion

The results of the experiments described in this paper show that supernatant fluids from cultures of malignant tumour cells uniformly and reproducibly caused depression of mouse peritoneal macrophage chemotaxis, while those obtained from rapidly replicating but non-malignant cells failed to depress macrophage chemotaxis. This confirms previous findings [1,5]. The above results also show that the ability to depress macrophage function, as measured by chemotactic migration, is not exhibited by rapidly dividing non-malignant cell lines, rat embryo fibroblast and baby hamster kidney fibroblasts. However, when malignant transformation occurred in such cells, supernatant fluids from cultures of these cells caused profound depression of macrophage function, suggesting that ability to depress macrophage locomotion is associated with the malignant state.

The tumours investigated in the present study were derived from humans, hamster and mice; they all depressed macrophage migration, indicating that this effect (i.e. depression of macrophage locomotion) is not species specific.

The present findings are consistent with reports from other workers of depressed mononuclear phagocyte function in tumour-bearing animals and humans. Synderman and Stahl [6] and Snyderman and Pike [7] showed that several animal tumours produced factors that inhibited macrophage accumulation *in vivo* and the chemotactic responses of macrophages and neutrophils (two separate factors) *in vitro*. Norman and Sorkin [8] produced similar findings in rats.

Otu *et al.* [1,5] proposed that malignant tumours release circulating factors that suppress macrophage functions. These factors are produced very early (24–72 h) after tumour implantation; early depression of macrophage function is an important step in allowing escape of tumours from host surveillance. The marked depression of macrophage chemotaxis shown by tumour cell lines in the present study is consistent with this hypothesis.

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Table 3. Effect of non-neoplastic cell lines on macrophage chemotaxis

Chemotactic factor	Distance in micrometres 250 µg	500 µg	% decrease in locomotion
REF	64.9 ± 0.1	70.7 ± 0.3	—
BHK	70.0 ± 0.1	79.6 ± 0.3	—
BHKT	42.3 ± 0.2	42.4 ± 0.1	33.3 and 44.0

Migration is expressed as the mean of 10 readings ± standard error.

Per cent decrease in locomotion = $\frac{\text{positive control} - \text{observed migration}}{\text{positive control}} \times 100.$

REF = rat embryo fibroblasts, BHK = baby hamster kidney, BHKT = baby hamster kidney polyoma transformed.

Negative control (Gey's solution only) = 41.0 ± 0.2.

Positive control (casein only) = 63.2 ± 0.3 and 75.1 ± 0.1 at 250 µg and 500 µg, respectively ($P < 0.001$ by Student's two-tailed *t*-test).

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