On the lymphatic spread of cancer

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Summary. Experimental demonstration that agents promoting cellular motility and/or vascular permeability enhance the spread of tumour to regional or distant lymph nodes. Tumour emboli appear to reach the regional glands via the lymphatic channels and the distant nodes by haematogenous route.

Lymphatic dissemination is determined i.a. by a) the motility of the tumour cell, and b) the permeability of vascular channels (bloodvessels and lymphatics). Animal tumours, in contrast to human cancers, rarely involve the lymphatic system[1].

In the present study, it will be shown that agents enhancing cellular motility and/or vascular permeability can induce lymphatic invasion by experimental cancer. The mechanism of tumour dissemination into the lymphatics will be discussed.

Materials and methods. Animals: White Wistar outbred rats weighing 150–180 g were obtained from the Hebrew University Animal Breeding Station, Jerusalem.

Tumours: A prostatic tumour, originally induced by intraprostatic injection of spermatozoa[2] was maintained by serial passage and used in these experiments. Tumour suspensions were prepared by mincing 1 vol. of tumour in 1 vol. of saline and passing it through a fine-meshed metal sieve. Amounts of 0.5 ml of this suspension were injected s.c. or into the thigh muscle.

Chemicals: Prostaglandin (PG) was obtained by courtesy of the Upjohn Co. (Kalamazoo) and prepared as a solution of 1.0 mg/ml by the method indicated by the manufacturers. Levamisole, received from Messrs Abic (Ramat Gan) was prepared as solution of 1.0 mg/ml saline. Trypsin (Difco, Detroit) was prepared as 0.2% solution in saline. Antiserum to (human) alpha, 2, macroglobulin (AMG) was obtained from Messrs Dako Immunoglobulins, Copenhagen.

Experimental procedure: 1 experimental group received 0.5 ml of PG solution simultaneously with the s.c. or i.m. inoculation of tumour with another dose of 1.0 ml of PG solution in situ 4 days later. A 2nd group received 4.0 ml of levamisole solution by i.m. injection of the 7th, 8th and 9th day after s.c. or i.m. tumour inoculation. A 3rd group was similarly treated with injections of trypsin solution. Antiserum to AMG (AMGAS) was given on the 7th day in amounts of 1.0 ml of undiluted serum administered into the contralateral thigh. The s.c. experimental group was sacrificed on the 12th–14th day. The experiments involving i.m. inoculations were terminated on the 17th–21st day, i.e. whenever the i.m. tumour was beginning to ulcerate. Lymph nodes and lungs were dissected out and weighed. The presence of tumour was verified by histological examination.

Histological technique: Tissues were fixed in formol saline and stained with haematoxylin eosin.

Results. PG tended to delay the take and growth of s.c. tumour. Thus, by the time the control tumour of the s.c. group had invaded the underlying muscle or had broken through the skin, the PG group only had small encapsulated s.c. growths. This was not the case with PG-treated i.m. tumours. These grew at the same rate as the i.m. controls. No adverse effects or no further tumour inhibition was observed with any of the agents used.

The table shows the incidence of lymphatic and pulmonary involvement in experimental and control animals. S.c. tumours tended to spread to the regional lymph nodes after treatment with these agents but never to distant glands, whilst i.m. tumour (unless ulcerating) appeared to spare the regional gland and to invade axial nodes. The weight of affected nodes varied from 0.3–3.7 g (weight of normal paraaortic node: 0.02–0.03 g) (figures 1 and 2). On histological examination of an inguinal gland, there were tumour cells in the cortical sinus. The paraaortic glands showed tumour emboli in arterioles and venules and tumour invasion into the medullary sinus (figures 3 and 4). The vessels of a tumour bearing lung had a characteristic perivascular cuffing with tumour cells (figure 5).

Discussion. The table shows that the a/m agents promote the spread of s.c. or i.m. tumour to the regional, viz. distant, lymph nodes. The PG-treated group formed an exception in that no regional gland involvement was observed. However, PG has been shown to impair the growth of s.c.[3] but not that of i.m. tumour, hence the difference in lymph node invasion.

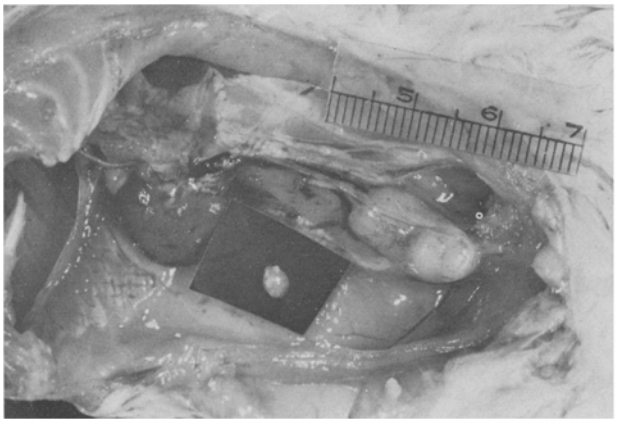

Fig. 1. Abdomen of rat bearing a 21-day thigh tumour and treated with trypsin. Inguinal node dissected out, no tumour but slightly enlarged. Paraaortic gland greatly enlarged and invaded with tumour.

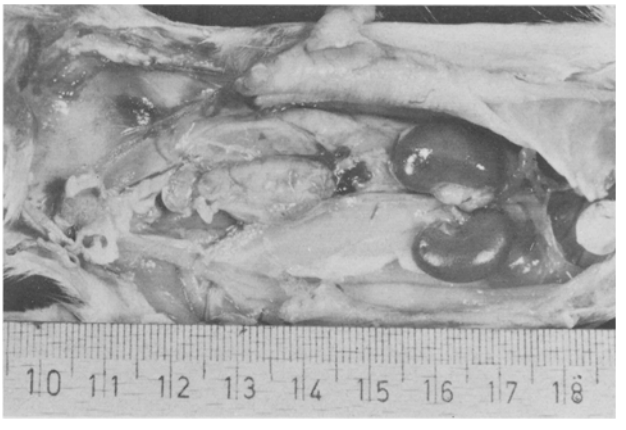

Fig. 2. Abdomen of rat carrying a 21-day thigh tumour and treated with levamisole. Chain of cancerous lymph nodes from renal plexus to aortic bifurcation.

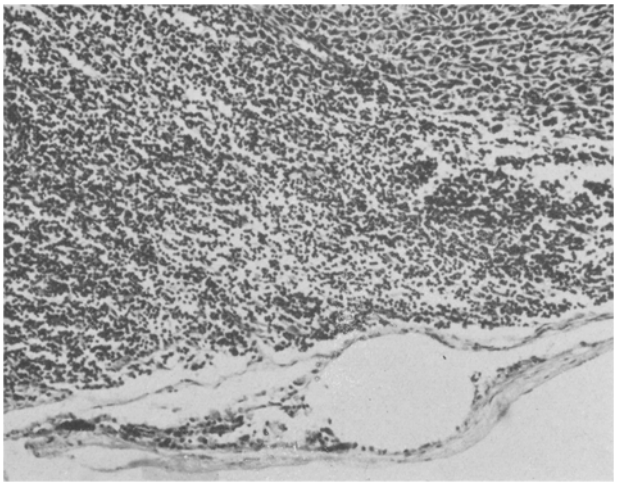

Fig. 3. Paraaortic lymph node of animal figure 1. Normal cortex, upper left quadrant (medulla) infiltrated with tumour cells. H.E. × 135.

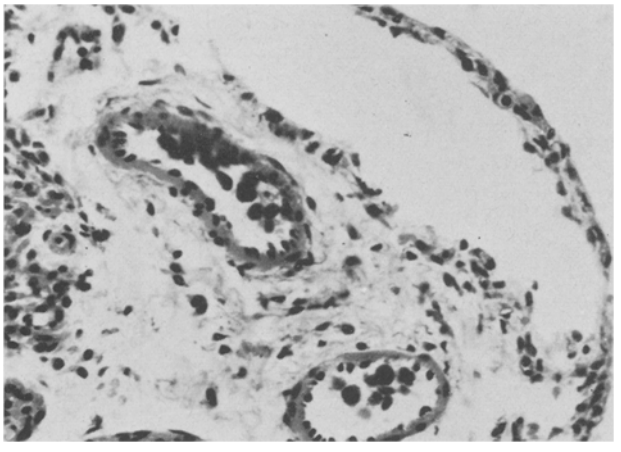

Fig. 4. Paraaortic lymph node of animal with thigh tumour treated with PG. Tumour cells in arterioles. H.E. × 350.

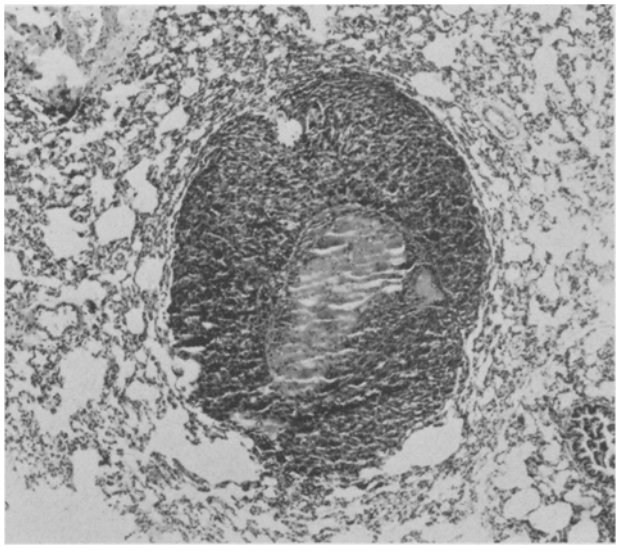

Fig. 5. Pulmonary vessel of above animal (fig. 4.) Perivascular cuffing with tumour cells. H.E. × 55.

Incidence of secondary tumours in lung and lymph nodes in animals carrying s.c. or i.m. primaries and treated with various agents

Group	Regional gland	Lung	Paraaortic gland
A s.c. tumour only	0/5	0/5	0/5
B s.c. tumour and PG	0/5	0/5	0/5
C s.c. tumour and levamisole	2/5	1/5	0/5
D s.c. tumour and trypsin	2/6	1/6	0/6
E i.m. tumour only	0/10	1/10*	1/10*
F i.m. tumour and PG	0/10	5/10	4/10
G i.m. tumour and levamisole	2/8*	4/8	3/8
H i.m. tumour and trypsin	2/10*	7/10	8/10
I i.m. tumour and AMGAS	0/9	7/9	2/9

* Infected ulcers skin. Abbreviations: PG, prostaglandin; AMGAS, antiserum to alpha, 2, macroglobulin.

Striated muscle is an immunologically privileged tissue devoid of lymphatics[4]. Animals bearing large thigh tumours and treated with the above agents had unaffected inguinal glands (as long as the skin was not involved) but frequently had large cancerous paraaortic nodes. It would appear, therefore, that in the latter instance, tumour emboli had reached the axial nodes by haematogenous route. This view is supported by our experimental findings: secondary tumour in the lung, presence of tumour emboli in the arterioles of the axial node and invasion of its medullary sinus.

As to the mode of action of these agents: we have no knowledge of the substances which specifically promote the migration of tumour cells. There are, however, agents known to enhance the migration of leucocytes. PG[5] and levamisole[6] belong to this group. It may be inferred that these substances exert a similar effect on other motile elements, e.g. tumour cells.

Tumour vessels and vessels draining tumour (pulmonary vessels) are believed to be lined by a protective layer of AMG[7]. The presence of this peptide on vascular endothel is thought to regulate its permeability to cellular elements, viz. tumour cells[8]. On this hypothesis, proteolytic agents (trypsin) or a specific antibody to AMG would digest, viz. inactivate such layer and render the vessel wall permeable to tumour cells. This proved to be the case. Similarly, bacterial proteases (streptokinase) might lyse endothelial AMG - hence glandular involvement in animals with abscesses or infected ulcerus.

It is in this manner that agents thought to enhance cellular motility (PG, levamisole) and/or vascular permeability (proteases, PG, AMG antiserum) could promote the spread of tumour to regional or distant lymph nodes.

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