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BRIEF COMMUNICATION

MACROPHAGES AND CARCINOMA CELLS MIGRATE AT THE SAME PACE TO THE LYMPH NODES

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To provide semiquantitative insight into the rate of migration of tumor cells to regional lymph nodes, we compared the transport of India ink loaded macrophages with Walker 256 (W256) carcinoma cells. In brief, W256 carcinoma cell fragments of 25mm^3 were implanted subcutaneously on both sides of the lower back at a distance of 2.5cm from the draining inguinal lymph nodes on either side in 26 female Sprague-Dawley (S-D) rats *(Fig. 1A)*. From day 0, two rats were sacrificed at 1/2h, 12h, 24h, 36h, 48h, and daily thereafter until day 10. The inguinal lymph nodes on both sides, the tumor implants and the tissue in between were excised, fixed en bloc, and examined by light microscopy to observe the daily advance of the tumor cells to the regional lymph nodes *(Fig. 1B)*.

A similar study was performed after instilling 50μl of India ink on both sides of the lower back in 26 other female S-D rats. The carbon particles were considered a marker for migrating histiocytes and traced by light microscopy by staining the nuclei with Kernechtrot. This method leaves the cytoplasm free of stain such that carbon particles within could be more easily recognized. The presence of small particles was confirmed by transmission electron microscopy.

FINDINGS

A. Tumor migration

From day 1 onward, the tumor implant site was edematous and infiltrated by a small number of macrophages. At day 2, the first malignant cells were seen in *sector 2 (Fig. 1B)*, a distance of 1mm from the main tumor mass. At the same time, isolated or small groups of morphologically intact tumor cells, some showing mitoses, were first detected in draining lymphatics more than 2cm from the implanted tumor *(sector 4; Fig. 1B)* and also in the marginal sinus of one groin lymph node. Many dilated lymphatic vessels were observed in the vicinity of the implants. At day 7 the draining groin nodes were filled with large groups of tumor cells in the marginal sinuses extending into the trabecular sinuses. At day 10 the invaded lymph nodes were largely replaced (1/2 or 2/3) by tumor cells primarily located in the sinuses with only local infiltration of the trabeculae. Examination of serial sections demonstrated that from 24h after implantation up to day 10 the main tumor mass was uniformly surrounded by isolated tumor cells and that detachment occurred continuously. Although the original size tumor mea-

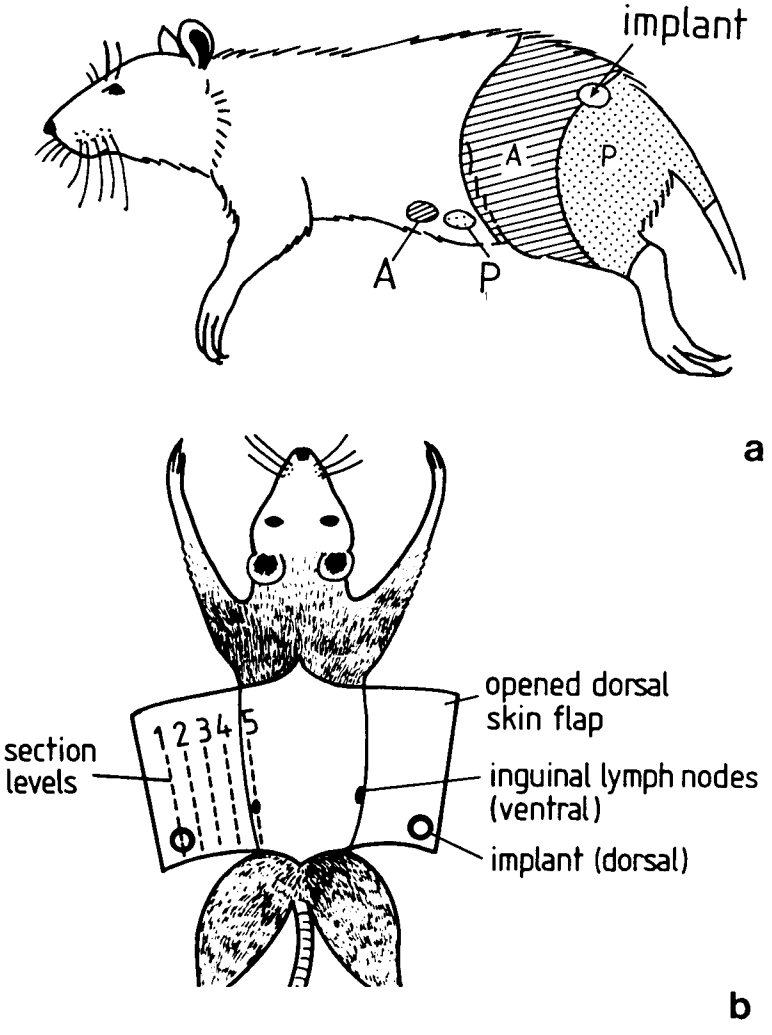

Fig. 1a. Lymphatic drainage areas of the individual anterior (A) and posterior (P) inguinal lymph node. Tumor cells or India ink was implanted on the low back as shown approximately 2.5cm from the regional groin nodes. 1b. Scheme showing the sampling technique of the implants, the inguinal lymph nodes, and the draining tissue between them subjected to light microscopy.

sured 2.5cm at day 10, the metastatic transport was irregular as summarized in *Table 1.*

B. Macrophage migration

At day 1 the site of India ink placement was surrounded by a moderate inflammatory reaction. At day 2 this reaction became more prominent and numerous macrophages containing carbon particles surrounded the black ink "mass" and could be traced in *sector 3* at a distance of 3.5mm from the main black blob. At day 3 isolated macrophages were present in dilated lymphatic vessels *(sector 4)* and detectable in the marginal and trabecular sinuses of the inguinal lymph nodes.

Table 1

Comparison of migration pattern (time sequence) of India ink loaded macrophages, and of Walker 256 (W256) carcinoma cells to regional inguinal lymph nodes. The first digit (numerator) is the number of involved lymph nodes, the second (denominator) the number of examined lymph nodes. The two adjacent lymph nodes of one side are counted together as one

Time h-hour d-day	½h	12h	24h	36h	48h	3d	4d	5d	6d	7d	8d	9d	10d
Ink (50μl)	0/4	0/4	0/4	0/4	0/4	3/4	2/4	4/4	4/4	4/4	4/4	4/4	4/4
W256 (125mg)	0/4	0/4	0/4	0/4	1/4	0/4	3/4	3/4	1/4	4/4	1/4	2/4	3/4

From day 5 until day 10 more than 200 macrophages with carbon particles were consistently counted in each lymph node section. As summarized in *Table 1*, the presence of macrophages was constant and maximal from day 5 onward.

COMMENT

The comparative transport dynamics of Walker 256 carcinoma cells and of macrophages show that they migrate at a similar pace and are able to reach within 3 days draining lymph nodes 2.5cm away. Nonetheless, macrophages seem to behave more regularly than tumor cells. That macrophages are attracted by the inflammatory stimulus of India ink is self-evident, but once these mononuclear cells have phagocytosed the carbon particles the chemo-attraction ceases. Cell transport rate to regional lymph nodes is influenced by augmented lymph flow secondary to local inflammation. Because both macrophages and detached tumor cells lie free in the interstitium, the mechanism of being carried along by the lymph stream probably accounts for a similar transport rate for each cell type. It is possible that active cell motion is also involved in migration toward regional nodes.

The metastatic process has been characterized as a cascade of events, one of which includes the transport migration rate of cells (1). Our findings support that the rate of displacement of tumor cells in a rapidly growing experimental tumor with high metastatic potential is similar to the migration rate of macrophages in response to an inflammatory reaction.

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