Experimental Studies on the Spread of Cancer in the Lymphatic System

IV. Retrograde Spread*

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This report is one in a series dealing with experiments designed to study the mechanics of the spread of cancer through the lymphatic system. In these experiments, viable transplantable tumor cells are injected directly into efferent lymphatics. Past work concerned the forward spread of cancer, from node to node and through the thoracic duct (2-4). Studies of lymphatic spread would be incomplete without consideration of retrograde spread, a form of metastasis occasionally seen in human cases (1). That is, cancer metastasizes not only from node to node as the lymph flows, but also to retrograde nodes, as well, in a direction counter to normal lymph flow. What pathways are used by tumor cell emboli while passing retrograde? Where do tumor cells first lodge in the retrograde node? Experiments were done to determine whether the retrograde spread of cancer in the lymphatic system could be reproduced experimentally as it is seen in man. If so, then the above questions may be answered.

MATERIALS AND METHODS

The transplantable V2 carcinoma was used in domestic rabbits. Cell suspensions were prepared by passing tumor fragments through a sieve into a mixture of balanced salt solution and serum. To determine whether retrograde lymphatic spread would occur, each rabbit was given injections of 1.0 cc. of tumor cell suspension into a pelvic afferent lymphatic--the right inguinal. This led to the development of tumor in pelvic lymph nodes. All rabbits were sacrificed 1-~ months later. The popliteal node of the same and opposite side was examined microscopically in semi-serial sections. The presence of tumor in the popliteal node would indicate retrograde spread of cancer from the pelvic nodes.

Before doing these experiments, it was first necessary to outline that part of the lymphatic system under study. 1.0 cc. of a 2 per cent solution of Berlin Blue was injected directly into the right inguinal lymphatic of ten rabbits, and the rabbits were examined immediately afterward. In all instances, the right inguinal lymphatic appeared as a blue line leading directly to blue pelvic nodes. From the nodes, the dye colored those retroperitoneal lymphatics which proceeded toward the diaphragm and ended in the thoracic duct. Retrograde staining of the popliteal efferent lymphatic or the popliteal node never occurred.

RESULTS

A suspension of V2 carcinoma cells was injected into the right inguinal lymphatic of 70 rabbits, and all rabbits developed cancer in the pelvic nodes. The right popliteal node revealed tumor foci in nineteen experiments. The left popliteal node was tumorous in but one instance. In five experiments retrograde spread had occurred within 40 days after tumor injection. Thus, retrograde spread of cancer in the lymphatic system occurred frequently, and as early as 6 weeks after introduction of tumor into the lymphatic system.

Location of early tumor growth in the retrograde node.—The popliteal nodes revealed one or more isolated tumor growths. These growths were situated exclusively in the subcapsular sinus region in fourteen of the twenty positive nodes (Figs. 1, 2). The other six nodes revealed tumor growths in both subcapsular sinus and medullary regions. In no experiment was tumor growth exclusively in the medulla. There were 75 early tumor growths altogether; 56 were in the subcapsular sinus and adjacent cortex, ten were in the medulla (Figs. 3, 4), and nine occupied both sites. Thus, when

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cancer spread retrograde in the lymphatic system, the early tumor growth was usually in the subcapsular sinus region, and the medulla was involved infrequently.

Lack of permeation in retrograde lymphatic spread.—Tumors may spread in the lymphatic system in two ways. Isolated tumor cell emboli may be carried in the lymph stream to the next node; or, tumor may grow as a solid cord in the lymphatic from one node to the next—a process called permeation. In the above experiments, tissue sections of lymphatics and adjacent tissue above and below the popliteal nodes were studied microscopically. Tumor was never found in the lymphatics. Thus, retrograde spread of tumor in the lymphatic system probably occurred by embolism rather than by permeation.

Potential pathways for retrograde tumor cell embolism.—Experiments were done to visualize the pathways used by tumor cell emboli while passing backward from pelvic nodes, and to see where emboli first lodge in the retrograde popliteal node. This was done by injecting stained, and hence identifiable, tumor cells retrograde into dilated popliteal efferent lymphatics, the only lymphatics normally connecting popliteal and pelvic nodes.

Retrograde injection was not possible in the normal, unobstructed lymphatic, probably because of valve action. It was first necessary to produce dilatation of popliteal efferent lymphatics by partial obstruction of the popliteal outflow. This obstruction was produced by tumor growth in the pelvic nodes. The inguinal lymphatic was injected with 1.0 cc. of viable V2 tumor suspension. One month later, pelvic nodes were enlarged with tumor, and popliteal efferent lymphatics were dilated.

Stained tumor cells were now prepared by fixing a suspension of V2 carcinoma cells in formalin and staining the cells blue with hematoxylin. In ten experiments, 1.0 cc. of a stained tumor suspension was injected retrograde into popliteal efferent lymphatics where they are superficial in the thigh, near the inguinal ligament (Chart 1). Following retrograde injection of the blue-stained tumor cells, popliteal nodes were removed, sectioned, and stained red with eosin only. Then the location of blue-stained tumor cells was easily ascertained by microscopic study of the node sections. The stained cells localized in the subcapsular sinus region in eight experiments (Figs. 5, 6); cells were found in both medulla and subcapsular sinus in the other two experiments. This result accorded well with that produced by spontaneously metastasizing tumor.

During retrograde injection of stained cells, the pathway taken by the cells became visible. The lymphatics showed the blue color of the contained cells (Chart 1, Fig. 7). From the injection site near the inguinal ligament, the popliteal efferent lymphatics showed as blue lines passing down toward the knee. However, the blue lines parted from the true popliteal efferents at the upper level of the knee joint, by-passed the popliteal node, and continued as channels in the superficial fascia down over the knee joint. The blue lines then joined the popliteal afferent lymphatics. The latter carried the stained cells upward to the popliteal node.

These experiments indicated an explanation for the high incidence of subcapsular sinus growths seen in the popliteal node when cancer spreads retrograde spontaneously from the pelvic node. Tumor cell emboli from the pelvic node may have followed the pathway outlined by the stained

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**Chart 1.**—Diagram of that part of lymphatic system used in experiments. (1) Pelvic afferent lymphatic. This lymphatic, in the inguinal fat, is the site where viable tumor cells are injected to produce pelvic node tumors. (2) Popliteal efferent lymphatics. High-level, retrograde injections of stained cells were made here. (3) Level just above the knee joint where popliteal effenter lymphatics emerge from the popliteal space and continue upward toward the inguinal ligament in the superficial fascia on the medial side of the thigh. (4) Collateral lymphatics. These channels become visible after retrograde injection of stained cells into dilated popliteal efferents. The collaterals are not stained in the normal animal by dye injection into popliteal afferent lymphatics in the paw. (5) Popliteal afferent lymphatics. (6) Popliteal node. (7) Pelvic lymph node.
cells, finally attaining the popliteal afferent lymphatics. Emboli in afferent lymphatics would then go forward and lodge in the subcapsular sinus of the popliteal node (3).

The collateral lymphatics.—What are these lymphatic pathways which by-pass the popliteal node and connect popliteal efferent and afferent lymphatics? Are they afferent lymphatics which dilate with lymphatic obstruction, lose valve function, and, hence, permit retrograde flow? If so, the channels should be injected when popliteal afferent lymphatics of rabbits are injected with dye in the normal, forward direction of lymph flow. The foot-pads of eight normal rabbits were given injections of a solution of Berlin Blue dye. This colored blue all the afferent lymphatics of the leg, and they led directly to the popliteal node (Fig 8). In no instance was there staining of lymphatics which by-passed the popliteal node. Thus, it is likely that the pathways for retrograde embolism are newly formed collaterals.

DISCUSSION

The demonstration that retrograde lymphatic spread of cancer occurs experimentally permitted an analysis of the mechanics involved. Cancer of the pelvic node metastasized to the popliteal node. In these experiments, tumor cell emboli were expected to pass retrograde through the popliteal efferent lymphatics, the channels connecting the popliteal and pelvic nodes. If this were true, then embolic lodgement and early tumor growth in the retrograde popliteal node should occur in the medulla, the site of origin of efferent lymphatics. Indeed, when stained cells were injected retrograde into efferents near the popliteal node, the cells localized in the medullary region of the nodes. Surprisingly, retrograde embolism of viable tumor from the pelvic node led to localization of early growths usually in the subcapsular sinus region of the popliteal node. This finding prompted experiments to reveal pathways taken by emboli while passing retrograde. It was found that emboli may leave the popliteal efferent lymphatics, pass through collaterals into popliteal afferent lymphatics, and then empty into the subcapsular sinus of the popliteal node. Tumor cells take circuitous channels in passing backward through the lymphatic system.

It is necessary to evaluate two other explanations of the unexpected localization of early tumor growth in the subcapsular sinus region of the retrograde node. First, tumor growth in the node may be caused by emboli arriving via the popliteal artery. Second, arterial emboli may have caused leg muscle tumors. These tumors could then metastasize via popliteal afferent lymphatics to the subcapsular sinus region of the node. Both of these explanations are very unlikely for several reasons. Metastasis from arterial emboli should affect both extremities equally. In the above experiments, retrograde spread of cancer occurred almost invariably on the side injected. Then, arterial tumor emboli to lymph nodes lodge and first grow at the junction of cortex and medulla—not in the subcapsular sinus region. Finally, there was no evidence of tumor growth in leg muscles. It is probable, therefore, that retrograde spread of cancer occurred within the confines of the lymphatic pathways outlined above.

SUMMARY

Experiments were done to study the mechanics of the retrograde spread of cancer in the lymphatic system. Cells of the transplantable V2 carcinoma were injected directly into pelvic afferent lymphatics of domestic rabbits. Tumor developed in the pelvic nodes and then metastasized retrograde to the popliteal node. Other experiments

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1 Personal observation.
Fig. 5.—Section of popliteal node demonstrating localization of stained tumor cell emboli in the subcapsular sinus region (arrow), following retrograde injection into the popliteal efferent lymphatic. The efferent injection was made in the thigh, near the inguinal ligament. Tumor cells were stained with hematoxylin before injecting. Node was stained with eosin only. Eosin. X12.

Fig. 6.—High-power view of popliteal node in Figure 5, demonstrating dark-stained cells in the subcapsular sinus region. Capsule and extracapsular fat of node are on the right. Cells were stained with hematoxylin before being injected. Node was stained with eosin only. Eosin. X170.

Fig. 7.—Collateral lymphatics which develop following lymphatic obstruction. View of the medial side of the right knee region of rabbit. The skin is removed. This rabbit had some lymphatic obstruction to the leg, produced by a previous injection of tumor cells into a pelvic afferent lymphatic and the subsequent development of pelvic node tumors. Then, stained cells were injected retrograde into the popliteal efferent lymphatic near the femoral artery at a site above the hemostat. The dark lines in the figure are lymphatics which take the color of the contained stained cells. Upper left arrow shows the collateral lymphatics which course downward in the superficial fascia over the knee joint and by-pass the popliteal node. Lower left arrow shows site where collaterals join popliteal afferent lymphatics. Lower right arrow reveals popliteal afferent lymphatics. Upper right arrow points to popliteal node (see Chart 1 also).

Fig. 8.—Injected popliteal afferent lymphatics. View of medial side of right leg and knee of normal rabbit. An aqueous solution of Berlin Blue was injected into the foot pad, and the popliteal afferent lymphatics were stained immediately. Lower arrow points to popliteal afferent lymphatics. Middle arrow points to site of turning of lymphatics posteriorly toward the popliteal node. Notice that no lymphatic above this point corresponds to the collaterals of Figure 7 which continue straight upward in the fascia over the knee joint. Upper arrow points to stained popliteal node. This picture demonstrates the normal popliteal afferent lymphatics and indicates that the collateral lymphatics of Figure 7 are not normal afferent lymphatics.
were performed to demonstrate lymphatic pathways used by tumor emboli while traveling retrograde. Stained tumor cells were injected retrograde into popliteal efferent lymphatics, and the lymphatics and popliteal node were then studied.

It is concluded that retrograde spread of cancer in the lymphatic system is seen experimentally, as it is seen in man. Such spread may occur as early as 6 weeks after the introduction of tumor into the lymphatic system. Early tumor growth in the retrograde node occurs usually in the subcapsular sinus region, and infrequently in the medullary region. Tumor cell emboli pass backward through circuitous collateral lymphatics which empty into afferent lymphatics of the retrograde node. These afferents then carry the emboli forward to the subcapsular sinus.

REFERENCES
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