Experimental Studies on the Spread of Cancer in the Lymphatic System

I. Effectiveness of the Lymph Node as a Barrier to the Passage of Embolic Tumor Cells*

IRVING ZEIDMAN AND JOANNE M. BUSS

(Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia, Pa.)

A method has been devised for studying the lymphatic spread of cancer experimentally. Briefly, in this method a suspension of cancer cells is injected into an afferent lymphatic vessel. Subsequently, cancer grows in the corresponding lymph node. The conditions under which further spread may occur are the subject of the present investigation.

This method has already yielded information on several questions which could not be easily answered by histologic examination of autopsy or surgical specimens; for example, where in lymph nodes are cancer cells first arrested? How long a time elapses before tumor cells escape from the node? Do tumor cells escape only after the node has been largely replaced by cancer, or do they escape before this occurs? Such questions on the effectiveness of the lymph node barrier are not only of biological interest but are also of practical importance to the human patient.

MATERIALS AND METHODS

The domestic rabbit was selected as the experimental animal, because satisfactory transplantable tumors are available and the lymphatics are of suitable size for injection. The tumors used were the V2 carcinoma and the Brown-Pearce carcinoma. Cell suspensions were prepared by passing tumor tissue through a sieve into a solution of equal parts of physiological saline and serum. The larger clumps of cells were permitted to settle for 15 minutes, and the supernatant, containing single cells and small clumps, was used for injections. In all experiments the popliteal lymph node was selected for study.

The rabbit was anesthetised with Nembutal.

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days after injection, the popliteal nodes were removed and histologic sections prepared. In these, early growth of tumor was found in the capsular lymphatics, the subcapsular lymph sinus, and the adjacent parenchyma. No tumor cells were seen in the hilar region.

When cells of the Brown-Pearce carcinoma were injected, results were the same as those obtained with V2 carcinoma (Fig. 4).

In the experiments just described, it seemed conceivable that individual tumor cells had escaped recognition, possibly after penetrating into the parenchyma. To rule out this source of error, stained and hence easily identifiable tumor cells were used in other experiments (2). Tumor cells were fixed in formalin and stained with iron hematoxylin. About 0.5 cc. of a suspension of stained V2 carcinoma cells was injected into the afferent lymphatics of the popliteal nodes of five rabbits. The rabbits were sacrificed immediately, and the popliteal nodes were examined. Grossly, the nodes revealed no signs of cancer at the time of sacrifice.

Microscopic section, stained with eosin for contrast, revealed localization of the blue stained tumor cells. The lobular pattern of localization of stained tumor cells. The lobular pattern of localization of tumor cell emboli is demonstrated.

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Effectiveness of the lymph node barrier.—The next series of experiments were designed to answer the question: How long is the lymph node an effective barrier to the spread of cancer? Even though in the experiments just described cancer cells had not been detected in the deeper parenchyma of the node, nor in the medullary sinus, yet the possibility remained that some viable cells could pass through a few days after arrival and escape into the efferent lymphatic. Accordingly, experiments were made to ascertain how soon viable cancer cells may escape the lymph node barrier. For this purpose, rabbits were injected with either the V2 carcinoma or the Brown-Pearce tumor. Injections of 0.5–1.0 cc. of a suspension of living cells was made into the lymphatics of the popliteal nodes. These nodes were removed surgically at various intervals, from 1 to 42 days after injection, and sections were examined microscopically for the presence of cancer cells. Only those animals having tumor cells in their popliteal nodes were included in the experiments. Four to 9 weeks after lymphadenectomy, the rabbits were sacrificed and autopsied. If cancer was then found anywhere in the body it was evidence that tumor cells had escaped from the lymph node before lymphadenectomy.

The results of these experiments are shown in Table 1. When lymphadenectomy was performed at any time during the first 3 weeks after injection, no cancer was found later either grossly or microscopically in the pelvic nodes. In those experiments in which the popliteal node had been excised between the 25th and the 42d day after injection, spread of cancer to pelvic nodes occurred in only two of fifteen animals. In these

FIG. 1.—Medial side of lower extremity with exposed popliteal space. Mandarin Black was injected into the popliteal afferent lymphatic, which appears as a black line running up from the lower margin of the photograph. The popliteal node is the black oval structure to the left of center. The efferent lymphatic is the black line coursing from the node up and to the right. The cut muscle below is the semimembranosus, and the reflected muscle above and to the right is the semimembranosus.

FIG. 2.—Gross appearance of popliteal nodes of two rabbits following injection of stained tumor cells into popliteal afferent lymphatics. The dark areas on the nodes are sites of retention of stained tumor cells. The lobular pattern of localization of tumor cell emboli is demonstrated.

FIG. 3.—Section through a stained zone in a popliteal node of Figure 2. The dark, stained tumor cells are localized in the subcapsular sinus and adjacent parenchyma. Hematoxylin and eosin. X 275.

FIG. 4.—Section through a lymph node removed 3 days after injection of viable Brown-Pearce carcinoma cells into the afferent lymphatic. The large tumor cells are seen in the two lymph vessels, the subcapsular sinus, and adjacent parenchyma. Hematoxylin and eosin. X 275.

TABLE 1  

<table>
<thead>
<tr>
<th>TIME OF POPPLITEAL LYMPHADENECTOMY (Days after injection)</th>
<th>NO. RABBITS WITH Pelvic Node Metastases</th>
<th>NO. RABBITS * LATER AT AUTOPSY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–3</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>10–20</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>25–35</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>36–42</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
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* This table includes only those experiments in which cancer was detected microscopically in the popliteal node. In twelve rabbits injected with V2 carcinoma and in fourteen with Brown-Pearce carcinoma no tumor was found in the popliteal nodes, and the rabbits revealed no signs of cancer at the time of sacrifice.
animals the popliteal nodes were almost completely replaced by tumor at the time of removal.

It is concluded that, under these conditions, lymph nodes are an effective barrier to the passage of cancer cells during the first 3 weeks after emboli are arrested in them.

Evidence suggesting that hematogenous spread from lymph nodes may occur.—The results of three experiments suggested a spread of cancer from the popliteal node into the blood stream. A popliteal node, harboring V2 carcinoma, was removed 25 days after injection. One month after lymphadenectomy, autopsy showed growth of cancer in the lungs, although the pelvic nodes contained no tumor. In the second instance, Brown-Pearce carcinoma was used, and the injected popliteal node was removed 1 day later; when this animal was sacrificed 6 weeks after lymphadenectomy, tumor was found in the adrenal gland. In a third experiment, with Brown-Pearce carcinoma, the popliteal node was removed 31 days after injection, and again tumor was found only in the adrenal gland at autopsy. In view of the absence of pelvic node metastases in these experiments, the possibility is suggested that tumor cells escaped from the popliteal node into the blood stream. However, the possibility of incidental injection of cells into venous channels during the lymphatic injection must be recognized. Also it might be that the pelvic node was bypassed through aberrant lymphatic channels.

DISCUSSION

A new approach to the study of the spread of cancer in the lymphatic system has been made possible by the simple and direct experimental method described. The injection of peripheral afferent lymphatics with tumor cells reproduced the pattern of metastatic lymph node cancer found in man. Thus, the spread of cancer from the popliteal to the pelvic node duplicated the chain-like pattern of lymphatic spread sometimes seen in human cancer. Likewise the experimental observation of the early localization of tumor cell emboli in the subcapsular sinus correlates well with the microscopic observations on surgical and autopsy specimens made by Willis (6), Walther (5), Gilchrist (4), and Coller et al. (1). These investigators observed that metastatic growth may be limited to the subcapsular sinus of lymph nodes.

It was indeed remarkable that in the majority of our experiments cancer did not extend beyond the popliteal node even after 6 weeks of intranodal growth. Removal of the cancerous lymph node was not followed by development of cancer elsewhere. These results strongly emphasize the effectiveness of the lymph node as a temporary barrier to the farther spread of cancer.

SUMMARY AND CONCLUSIONS

A method has been devised to study the spread of cancer in the lymphatic system experimentally. In this method, cells of Brown-Pearce carcinoma or V2 carcinoma are injected into popliteal afferent lymphatics. Cancer then develops in the corresponding lymph node and only after considerable growth subsequently spreads to the next node in the chain.

It is concluded that tumor cell emboli in afferent lymphatics are arrested in the subcapsular sinus of one or more lobules of the corresponding lymph node. In this location early growth occurs. The tumors do not spread to the next node for at least 3 weeks after initial arrest of the emboli, and are usually retained in the first node for a much longer time.

REFERENCES

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