Fate of Circulating Tumor Cells

III. Comparison of Metastatic Growth Produced by Tumor Cell Emboli in Veins and Lymphatics[1]

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SUMMARY

Experiments were designed to determine the relative ability of tumor cell emboli in blood and lymph to produce metastases. The transplantable V_2 carcinoma was used in domestic rabbits. Equal volumes of the same suspension were injected into veins of some rabbits and into lymphatics of others. The rabbits were sacrificed later and the incidence of metastasis was determined. Under these circumstances, metastases occurred with significantly greater frequency in rabbits receiving the injection I.V. than in those which received the tumor into lymphatics. The experimental results suggest that some tumor cell emboli are destroyed in the lymph node.

Primary cancers frequently metastasize to distant organs via 2 routes—veins and lymphatics. Venous spread yields metastases in parenchymatous organs, particularly lungs—and lymphatic spread is followed by metastatic growth in regional lymph nodes. Spread via both routes occurs by tumor embolism, and infrequently lymphatic spread also occurs by permeation (3). To date, there has been no attempt to compare the fate of tumor emboli introduced into veins and lymphatics. Because of the high incidence of metastatic cancer in lung and lymph node, it has been tacitly assumed that the tumor cell emboli in venous or lymphatic channels are equally capable of yielding metastases. Experiments were designed to evaluate this assumption. Equal numbers of tumor cell emboli were injected into veins of some rabbits and directly into lymphatics of others (6). The incidence of metastasis was determined later at autopsy. Surprisingly, a higher incidence of metastasis occurred in animals receiving tumor cell emboli I.V.

MATERIALS AND METHODS

The transplantable V_2 carcinoma was used in domestic rabbits. Suspensions of tumor cells were made by passing fragments of tumor through a sieve into a mixture of balanced salt solution and serum. Clumps were removed by centrifugation. Identical volumes (0.05 ml) of the same suspension were injected I.V. in some rabbits and into the inguinal lymphatic of others. Injections into both groups were performed slowly over a period of 2 min. A total of 80,000 tumor cells was present in each inoculum. Rabbits from each group were sacrificed 6–30 weeks after they received the injections and complete autopsies were performed. Lungs of rabbits receiving injections I.V. were thoroughly studied both grossly and microscopically, because venous emboli of V_2 carcinoma cells are immediately arrested in the lungs in most instances (5). Also, complete studies of the pelvic nodes were made in the intralymphatic series; these nodes receive the inguinal lymphatic, and V_2 tumor emboli in lymphatics are arrested by the first node encountered (6).

RESULTS

Of 49 rabbits receiving lymphatic injections of tumor suspension, 8 developed lymph node metastases (Fig. 5). Those without tumor in nodes had no metastases elsewhere. In contrast, when 51 rabbits received an equal volume of the same tumor suspension I.V., 25 developed lung metastases (Fig. 4). The difference in incidence was significant ($P < 0.01$). Thus, when tumor cell emboli were introduced into veins and lymphatics, a higher incidence of metastasis resulted from emboli in the blood stream.

Metastases occurred infrequently beyond the target organs—the lung or lymph node. The distribution and time of occurrence of these distant metastases suggested that they were the result of embolic spread from metastatic lesions in the target organs. Thus, in the lymphatic series, 7 of the 8 positive rabbits had tumors limited to

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regional lymph nodes and 1 revealed several lung metastases in addition. The latter rabbit was sacrificed 24 weeks after injection. Likewise in the I.V. series, 3 of the 25 rabbits with lung tumors had metastases elserwhee. The sites were the peribronchial lymph nodes, liver, and kidney. All 3 rabbits were in the series sacrificed 18–30 weeks after injection. In the I.V. series, as in the lymphatic series, all rabbits with negative target organs had no metastases elsewhere.

It may be contended that these results simply reflect a possible slow growth rate of metastatic tumor in lymph nodes as compared with the lungs. The data contradicted this possibility. Animals examined 6–17 weeks after intralymphatic injection revealed the same incidence of node growth as those autopsied 18–30 weeks after injection. Therefore, the difference in incidence of metastasis in lung and lymph node was independent of the duration of tumor growth.

Comparison of tumor growth following S.C. and I.V. injection.—Tumor cells introduced I.V. are well dispersed before ultimate arrest in lung capillaries. In contrast, the injection of tumor cells into lymphatics is followed by localization and clustering of cells in one or a few foci of the draining node (6, Fig. 3). It seemed possible that the low incidence of metastasis in the lymphatic series could be related to this sieving action of the node in the following way. Suspensions of tumor cells derived from solid tumors contain some necrotic cells (4). During the localization of the injected suspension in the node, the necrotic cells may surround the viable ones, thereby preventing diffusion of metabolites. In contrast, following I.V. injection, cells disperse and then localize in the lungs as single cell units with no block to nutrition. Hence, the higher incidence of tumor metastasis in the lungs.

The above explanation of the differences in incidence of metastasis in lungs and lymph nodes may be evaluated experimentally. It is necessary to compare incidence of metastasis when tumor cells are injected into veins and into another site where aggregation of emboli occurs in a fashion similar to that seen in lymph nodes. It seemed possible that such aggregation might occur S.C. To test this, a suspension of stained tumor cells was injected S.C. in 5 rabbits and the margin of the resultant bulge was outlined. Within 2 hr., the size of the bulge decreased appreciably. Then the animals were sacrificed, the injected site was inverted, and the area occupied by the stained cells was observed. In all instances the final area occupied by the injected cells was considerably less than the original area occupied by the inoculum (Figs. 1, 2). Microscopic sections revealed clumping of cells. The same results were obtained in other similar experiments with viable tumor cell suspensions. Hence, the aggregation of cell emboli S.C. approximated more closely the clumping of emboli in the lymph node as compared to the dispersion of emboli following I.V. injection.

Then, identical volumes of a dilute suspension of viable V_2 carcinoma cells were injected S.C. in some rabbits and I.V. in others. Equal numbers of rabbits from each series were sacrificed at intervals of 6–26 weeks after injection. Rabbits receiving S.C. injections revealed local growth in 20 of 33 instances. Rabbits injected I.V. developed lung tumors in 18 of 34 instances. Thus, the incidence of tumor growth was practically identical in both series. These results suggest that the low incidence of tumor growth following intralymphatic injection may not be related to the state of aggregation of emboli within the node.

DISCUSSION

The above experiments with V_2 carcinoma clearly demonstrate that tumor cell emboli produce more metastases in lungs than in lymph nodes. However, the applicability of these results to the behavior of spontaneous cancer may seem controversial. For example, the use of a *transplantable* tumor immediately suggests the possibility that the observed lower incidence of lymph node metastasis is due to destruction of trapped tumor cells by an immune reaction—a reaction probably lacking in spontaneous cancer. Yet, this immunologic explanation appears faulty. The elements required for an immune response appear in the efferent lymph and serum at about the same time as they appear in the lymph node (2); therefore, emboli trapped in the lungs should be exposed to a similar immune response. Even more important in this regard are the findings of DeLong and Coman, who measured the growth rate of V_2 carcinoma implants in a variety of organs (1). The largest growths appeared in the spleen, an organ which, like the lymph node, is a major source of elements needed for the immune response. This evidence seems to weaken the contention that an immune response accounts for the low incidence of node metastasis. With or without this potentiality of immune response, the lymph may be a relatively noxious milieu for tumor cell emboli. Present work is aimed at exploring these possibilities.

In the past, most investigators have vigorously denied the suggestion that tumor cells are destroyed in lymph nodes (3). The denial was based on the observation that carcinomas frequently metastasize to lymph nodes. However, this observation does not vitiate the possibility that *some* tumor emboli may be destroyed in nodes. It is also conceivable that such destruction is limited to the few tumor cell emboli which arrive initially; later repeated embolic bombardment overcomes this destructive tendency. Either of these explanations seems to reconcile any presumed disparity between the experimental observations cited here and the observation in man of a high incidence of lymph node metastasis.

REFERENCES

1. DELONG, R. P., AND COMAN, D. R. Relative Susceptibility of Various Organs to Tumor Transplantation. Cancer Res., **10**: 513–15, 1950.
2. EHRICH, W. E., AND HARRIS, T. N. The Formation of Antibodies in the Popliteal Lymph Node in Rabbits. J. Exptl. Med., **76**: 335–48, 1942.
3. WILLIS, R. A. The Spread of Tumors in the Human Body, pp. 18, 22, 23. St. Louis: C. V. Mosby Co., 1952.
4. ZEIDMAN, I.; MCCUTCHEON, M.; AND COMAN, D. R. Factors

Affecting the Number of Tumor Metastases. Experiments with a Transplantable Mouse Tumor. Cancer Res., **10**: 357–59, 1950.
5. ZEIDMAN, I., AND BUSS, J. M. Transpulmonary Passage of Tumor Cell Emboli. Cancer Res., **12**: 731–33, 1952.
6. ———. 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. Cancer Res, **14**: 403–05, 1954.

FIG. 1.—Outline of bulge produced by the S.C. injection of suspension of stained tumor cells in a rabbit. A suspension of V_2 carcinoma cells was fixed in formalin and stained with hematoxylin. Then 1.0 ml of suspension was injected S.C. The raised area of the injection site was then outlined with a black marking pencil.

FIG. 2.—Concentration of stained tumor cell emboli following S.C. injection. The rabbit of Fig. 1 was sacrificed 2 hrs. after injection. The skin of the injected site was removed, and the subcutaneous side of the skin was photographed as Fig. 2. There is a single black clump of stained tumor cells. The area occupied by the clump is considerably smaller than the area of the original injection site inside the circle of Fig. 1. As the fluid portion of the inoculum is absorbed, there is marked clumping and concentration of the cellular portion.

FIG. 3.—Localization of stained V_2 carcinoma cells in lymph nodes. A suspension of stained cells was injected into popliteal afferent lymphatics of 2 rabbits. The popliteal lymph nodes were removed shortly afterwards. The dark areas are sites of retention of stained tumor cells. Tumor cell emboli are concentrated in the lymph nodes as in the subcutaneous site.

FIG. 4.—Metastasis of V_2 carcinoma in the lung following I.V. injection of tumor suspension. Metastatic tumor is on left. H. & E., × 150.

FIG. 5.—Metastasis of V_2 carcinoma in the lymph node following injection of V_2 carcinoma suspension into afferent lymphatic. Tumor occupies lower half of picture. H. & E., × 150.