The Time Interval between Tumor Inoculation and Metastatic Spread to the Lymph Nodes*

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Little is known about the time when a malignant growth may first release tumor emboli into the lymphatic system. The problem can be investigated by employing the technic of anterior chamber inoculation, since the primary tumor may be readily destroyed by eye enucleation or x-radiation. Mouse tumor C-1300 appeared suitable for this purpose, since it metastasizes to the cervical lymph nodes of all inoculated animals of a susceptible strain. Because the metastases remain confined to the cervical nodes, it is unlikely that spread has occurred by other than lymphatic channels. It was the purpose of this study to determine when the tumor first metastasizes from the eye to the regional lymph nodes. Clearly, the results apply only to the tumor and host strain studied.

MATERIALS AND METHODS

Mouse tumor C-1300 (neuroblastoma) was injected into the anterior chamber of ABC mice of either sex weighing 15–22 gm. The technic of transplantation has been described previously (1).

Four methods were used to detect the presence of metastases in the cervical lymph nodes.

1. **Eye enucleation.**—At definite intervals after transplantation, the affected eye was enucleated by lifting and incising the conjunctiva and by severing the eye from its lateral and posterior attachments without undue pressure on the eye to prevent spillage of tumor cells into the periorcular structures. The animals were observed over a period of 60 days for the development of cervical lymph node enlargement.

2. **X-ray therapy.**—3,000 r/air, (100 kvP, 10 ma., 1.0 mm. Al filter, HVL 2.0 mm. Al, FSD 21 cm.) were administered as a single treatment through a 0.8-cm. field surrounding the orbit on the 4th, 5th, 6th, 8th, 10th, 12th, and 15th day, respectively. Lymph node enlargement was recorded as in above paragraph, method no. 1.

3. **Lymph node transfer.**—At definite intervals after tumor transplantation to the anterior chamber, animals were sacrificed. The four cervical lymph nodes were dissected out and bisected, and two halves each were inoculated into each flank of recipient mice; two recipient mice and four inoculation sites were used per sacrificed mouse. The development of subcutaneous tumors in the recipient mice was recorded.

4. **Histologic observation of lymph nodes.**—Animals were sacrificed as in method no. 3. The cervical lymph nodes of a small number of animals were divided. One half of each node was used for histologic sections; the other half was used for subcutaneous transplants. This method was abandoned, since it proved to be the least sensitive method for detection of the presence of metastatic tumors in the cervical lymph nodes.

Only those animals in which the local tumor in the eye disappeared completely following x-ray therapy or enucleation were utilized. All animals in which the local tumor recurred in the orbit were discarded, since metastases could have arisen from these tumors.

RESULTS

The results are shown in Table 1. The two methods of local eradication of the tumor are combined, since the figures were very similar. Metastatic growth was not seen in any animal treated prior to the 4th day.

A very small number (3 per cent) developed cervical metastases as early as the 4th day following tumor transplantation. Growing tumor could be demonstrated in the lymph nodes of all mice by the 12th day. The incidence of metastases was directly proportional to the time elapsed following transplantation.

The data obtained by the lymph node transfer method are comparable. The earliest demonstration of metastases was achieved on the 6th day following tumor transplantation. All animals had metastases demonstrable by this method on the 12th day. However, transfer on the 15th day resulted in only five of seven positive nodes.

Histologic study revealed tumor in cervical lymph nodes no earlier than 15 days after eye inoculation.
DISCUSSION

Following transplantation in the anterior chamber tumor growth passes through three stages according to Towbin (2):

1. "Suspension"—In this stage, the tumor floats unattached in the fluid of the anterior chamber.
2. "Nidation"—When the tumor cells are integrated with the vascular system of the host.
3. "Growth"—When expansive proliferation of the organized tumor tissue occurs.

When the fate of the tumor cells after anterior chamber transplantation was studied by means of daily serial sections of the eye,1 the first evidence of tumor growth of the cells was found in some animals on the 2d day. On the 3d day, tumor metastases invasion of the periscleral space has already taken place.

It is easy to understand why local eradication of the tumor in the orbit appears to be the most accurate method and makes possible the demonstration of metastases 2 days earlier than the "lymph node transfer" method. The trauma caused by lymph node transfer and the probability of detecting tumor cells in a few sections through these nodes would be expected to be less accurate than other methods.

SUMMARY

Following anterior chamber transplantation of mouse tumor C-1300 into ABC mice, the development of cervical lymph node metastases was observed by various methods. These were eye enucleation, x-ray therapy and observation of cervical lymph node enlargement, transfer of cervical lymph nodes into the flank of susceptible mice, and histologic observation of cervical lymph nodes.

The occurrence of metastases coincides with invasion of the choroid and sclera as demonstrated by serial sections of the eye following transplantation.

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REFERENCES


1 E. J. Eichwald, unpublished data.
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