CHARACTERISTICS OF THE METASTASIS OF CRM-1 STRAIN OF RHABDOMYOBLASTOMA

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Cellophane tumors were first produced by Oppenheimer and Stout [7] in 1948. By 1953, the authors had already counted about 150 primary cellophane tumors, of which only two were benign [8]. The remaining tumors were defined as various sarcomas, certain of which proved to be transplantable to five generations. However, we did not find indications of metastasis of cellophane tumors when they were transplanted, although in their first work [7] the authors noted the appearance of metastases in three cases out of 23 of primarily induced sarcomas. The problem of metastasis is of primary importance for determining the malignant nature of cellophane tumors. It is known that only a negligible number of transplantable tumors metastasize. These include Claudman melanoma, nonpigmented melanoma 91A, osteogenic sarcoma 112, Jensen rat sarcoma, and certain other tumors. In other transplantable tumors metastases are not detected in the norm or their percent is extremely negligible (Crocker rat sarcoma, Walker sarcoma, Rous sarcoma, etc.) [6].

A. N. Studitskii [2, 3] obtained for the first time a neoplastic transformation of striated muscle (gastrocnemius of rats) when it was wrapped with cellophane. The tumor is a typical rhabdomyoblastoma, in the terminology of Rakov [1], which is transplanted in 95-100% of the cases at the site of the extirpated gastrocnemius and has already experienced 27 generations. The malignant nature of this tumor, in addition to its rapid infiltrating growth and high transplantability, is proved by the presence of metastases in most rats with a month-old tumor.

Taking into account the insufficiency of the data in the literature on metastasis of myomas [1, 6], we decided to observe the development of metastases formed in cellophane rhabdomyoblastoma.

METHOD

Metastases of cellophane rhabdomyoblastoma CRM-1 were obtained for the first time during development of the 8th generation tumors. Starting from this period they were present almost in all rats which lived more than a month. The main tumor on the leg by this time was already subjected to severe necrosis, and an exudate was continuously pouring from it; the rat itself was very emaciated. Metastases in the form of solid nodules ranging from 2-3 mm to 1-1.4 cm in size were usually detected on the mesentery on the dorsal side, sometimes in close relation with the ureters or with the great vessels. Tumor cells were elicited in the histological examination in the lymphatic nodes of this region.

The metastases and lymphatic nodes of the abdominal cavity were fixed in Zenker's solution. Paraffin sections, 4-6 μ thick, were stained with Heidenhain's iron hematoxylin with Mallory counterstain, azocarmine with Mallory counterstain, and the DNA reaction was carried out for a better elicitation of mitoses.

RESULTS

The metastases were covered by a connective-tissue sheath which in the histological examination was rather loose in certain places and contained an appreciable number of blood vessels (Fig. 1). The tumor cells singly or in small groups sometimes penetrated deep into the sheath. Such metastases were characterized by numerous lymphocytes along the periphery. The lymphocytes, however, did not wholly encircle the metastasis, but formed

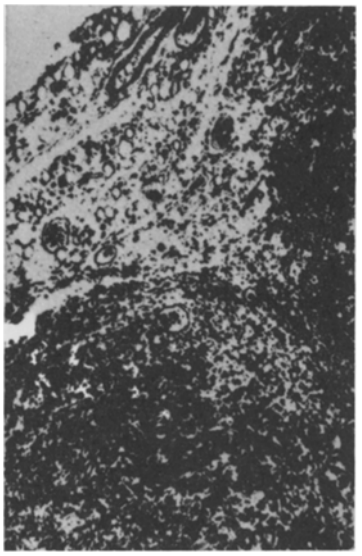

Fig. 1. General view of metastasis surrounded by a loose connective-tissue sheath. Peripheral infiltration by lymphocytes. Photomicrograph. Zenker. Iron hematoxylin. Objective 10 × ocular 8 × .

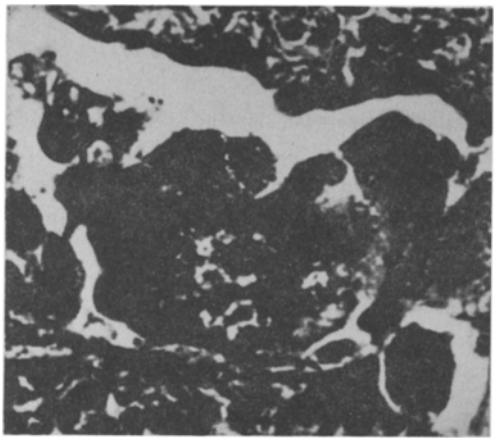

Fig. 2. Multinuclear giant cell. Photomicrograph. Zenker. Iron hematoxylin. Objective 40 × ocular 8 × .

accumulations where it was attached to the animal body. The lymphocyte accumulations can put out tapered protuberances.

The fibers of the connective tissue penetrate the entire metastasis, breaking it into separate pieces. The finer fibers entwine the individual cells.

As for the cellular composition of the metastases themselves, it is interesting to note here a certain difference in the structure of the metastases of the earlier generations from the later. The metastases of the early generations in our case (8th and 13th) are quite similar in structure to the main tumor. They contain numerous myoblastic-type cells, which form clearly visible strands which project in various directions. The nuclei of the myoblasts are large, light, and the mitotic patterns can be seen. The myoblasts frequently form symplasms. Giant cells are encountered in a large quantity, being more abundant along the periphery of the metastasis than in the middle portion (Fig. 2). Metastases of the later generations (Fig. 3), unlike the main tumor and the early metastases, almost entirely consist of large round cells with enormous nuclei, several of which can be in one cell. Mitoses are frequently encountered in the giant cells; they can be multiple; fragmentation of chromosomes and the formation of bridges were noted.

In certain cases the middle part of the metastasis had already started to undergo necrosis. The tumor cells became appreciably fewer, the nuclei in them were subjected to pyknosis, distinct cell boundaries could not be seen. The connective tissue greatly expands in such places.

The metastases affecting the lymphatic nodes have their own characteristics. All stages of lesion of the lymphatic node by tumor cells can be traced on preparations. Individual giant cells appear in the marginal sinuses of the node during the early stages. Later their number markedly increases evidently owing to division of the cells already present and the entrance of new ones. At first the giant cells form isolated, very compact accumulations along the periphery of the node. Then these accumulations increase, merge, and at late stages entirely surround the lymphatic node. Individual strands of tumor cells penetrate the node, occupying an ever greater area. Finally, the entire lymphatic node is replaced by tumor cells and only in the middle of the node do a few lymphocytes remain.

These data demonstrate the high degree of malignancy of the CRM-1 strain of rhabdomyoblastoma [2, 3], originally obtained by wrapping the gastrocnemius with cellophane. Along with the high number of takes in transplantation and the intensive infiltrating growth, strain CRM-1 beginning with the 8th generation acquired the property to yield metastases of a structure typical for this type of tumor. The manifestation of the capacity to metastasize, as well as certain changes in the microstructure of the metastases (simplification of structure and further dedifferentiation) indicate a further increase in the malignancy of the strain.

Observations of the development of neoplastic tissue in the lymphatic nodes, as well as of the lymphoid infiltration of the metastases, showed that the development of the transplanted tumor causes a specific reaction of the immunogenic tissue. It is known that foreign tissue transplanted to an organism causes the reaction of transplantation immunity [4, 5] which is accomplished by lymphocytes through their direct contact with the foreign tissue. The

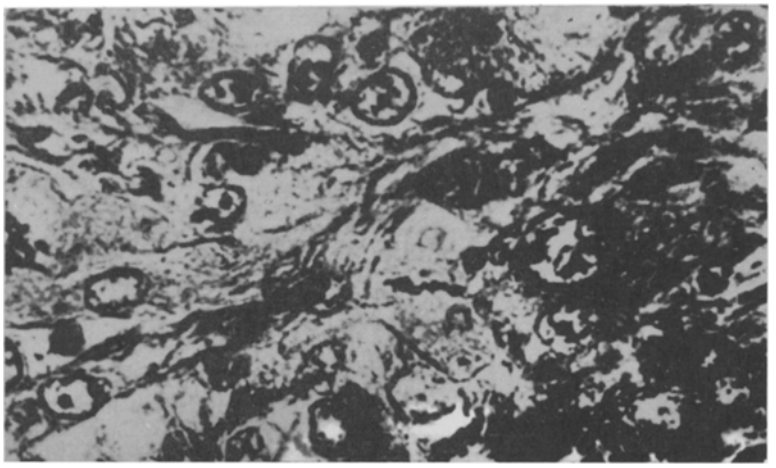

Fig. 3. Structure of metastasis, giant cells, myoblasts, mitoses of giant cells. Photomicrograph. Zenker. Iron hematoxylin. Objective 25 × ocular 8 × .

immunological reactivity to neoplastic tissue is inadequate to resist the growth of the foreign tissue, by virtue of which the growth of transplanted tumors upon homotransplantation in passages is possible. The development of neoplastic tissue in the lymphatic nodes, which is completed upon destruction of the lymphoid tissue, demonstrates that the neoplastic tissue counteracts the protective reaction of the immunogenic tissue. However, the development of a lymphocyte bulwark around the metastases and lymphoid infiltration of the neoplastic tissue in metastases indicates that growth of the transplanted tumor to some extent stimulates the immunogenic tissue to develop transplantation immunity, which acts against the metastases, retarding their growth in comparison with the transplanted neoplastic tissue but proves to be inadequate for its destruction.

SUMMARY

The paper deals with metastasis of the transplantable CRM-1 rhabdomyoblastoma obtained as the result of enwrapping the gastrocnemius in cellophane membrane. Metastases into the mesentery and lymph nodes of the abdominal cavity appear after a 30-day development of the principal tumor. The noted lymphoid infiltration of metastases, their slower growth as compared to the principal tumor, as well as affecting of the lymph nodes, testify to specific reactions of the recipient's immunogenic system in respect to the tumor tissue.

LITERATURE CITED

1. A. I. Rakov, In: Ten Years of Activity of the Leningrad Oncological Institute [in Russian], Leningrad (1938), p. 258.
2. A. N. Studitskii, Dokl. AN SSSR (1962), 146, No. 3, p. 724.
3. A. N. Studitskii, Zh. obshchei biol. (1962), No. 3, p. 176.
4. A. Ya. Fridenshtein, Uspekhi sovr. biol. (1961), 52, No. 3, p. 291.
5. M. Gashek, Uspekhi sovr. biol. (1961), 51, No. 2, p. 153.
6. E. Kaudri, Cancer Cells [in Russian], Moscow (1958).
7. B. S. Oppenheimer, E. T. Oppenheimer, and A. P. Stout, Proc. Soc. exp. Biol. (N. Y.) (1948), V. 67, p. 33.
8. B. S. Oppenheimer, E. T. Oppenheimer, and A. P. Stout et al., Science (1953), V. 118, p. 305.