Studies in Immunization Against a Transplantable Cerebral Mouse Glioma*

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The problems of management of the patient with a malignant intracranial glioma have remained essentially unchanged during the past few decades, although advances in roentgenography and electroencephalography have in many cases made earlier diagnosis possible. Most neurosurgeons would accept Zülch's statement: "Glioblastomas and medulloblastomas recur without exception even after apparently total extirpation and x-ray therapy. All apparent exceptions to this rule were later shown to have been an error of classification."

The immune mechanisms of the central nervous system, particularly in relation to transplantable tumors and homografts, have not been studied extensively. The results of current treatment of malignant gliomas, and the opportunities to study the immune mechanisms of the central nervous system should encourage investigation of the effects of immunization on transplantable cerebral gliomas.

It has been found that growth of a subcutaneously implanted glioma can be inhibited by previous immunization with mixtures of glioma and adjuvant in an isologous system. Further investigation of this phenomenon, using intracerebral implantation of a glomatous pellet as the challenge, was thought worth while, for a number of reasons: (1) Possible interference of certain factors, such as genetic divergence of tumor and host-cell lines after multiple transfers, might be reduced, since the brain is one of the "immunologically privileged" sites of the body. (2) The mechanism of stimulation of the immunity of the host and the way the immune factors reach the tumor across the blood-brain barrier might be clarified. (3) Growth of the tumor, with the resultant increasing intracranial pressure, might provide a sharp end point, with good criteria for clinical evaluation of inhibition. The experiments have shown that immunization with mixtures of glioma and adjuvant before intracerebral implantation of a pellet of glioma inhibited the growth of the tumor in an isologous system.

Materials and Methods

The mice used were the inbred strain C57BL/6J, supplied by the Roscoe B. Jackson Memorial Laboratory. The glioma was an ependymoblastoma induced in this strain by intracerebral implantation of a pellet of methylcholanthrene by Zimmerman and Arnold.

The tumor was carried through more than 100 subcutaneous transfers; it is accepted by almost all the mice of this strain, and even after many transfers still retains the original histologic characteristics of an ependymoblastoma (Fig. 1). The tumor is made up of small nests and cords of medium-sized, pleomorphic, polyhedral cells with poorly defined membranes and moderate quantities of eosinophilic cytoplasm. The nuclei are oval or round, with prominent mitotic figures. There are some areas of necrosis in the tumor, and the surrounding parenchyma is invaded occasionally. Inflammatory cells at the periphery of tumor have not been seen.

The subcutaneously maintained glioma was removed and cut into 1 c.m.m. pellets fitting into the lumen of a modified 18-gauge needle used for the intracerebral implantation. The trocar of the needle regulated roughly the size of the pellets of tumor implanted. Under intraperitoneal anesthesia with barbiturate, a burr hole was made with a dental drill in the right side of the skull, and the pellet of tumor was implanted in the right

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FIG. 1. Photomicrographs of a transplantable glioma of the mouse. (a and b) Nests and cords of pleomorphic, polyhedral cells. X100; X180. (c) Oval or round nuclei with prominent mitotic figures. X400. (d) Areas of necrosis in the tumor. X40.

cerebral hemisphere. The mixture of immunization consisted of equal parts of tumor, by wet weight, and solution of physiologic saline homogenized in a glass homogenizer with twice the total volume of Freund's complete adjuvant.* For the control immunizations, brains of normal C57BL/6J mice were substituted for tumor in the emulsion.

For each inoculation, 0.1 ml. of the mixture of immunization was injected into each of two intradermal sites. Several schedules of immunization were tried in order to determine which was the most effective: one, two or three inoculations given before, simultaneously with, or after intracerebral implantation of tumor. One group was given 0.5 ml. Hemophilus pertussis vaccine diluted with physiologic saline; 1:5, intraperitoneally in addition to ascertain whether this would enhance immunization. The results are shown in Table 1. All mice were sacrificed when death seemed imminent; the brains were removed, and examined histologically.

Results

In the unimmunized mice, the intracerebrally implanted pellet of glioma grew large enough within 2 to 3 weeks to produce obvious general or neurologic signs, such as general weakness, curling up in the corner of the cage, hemiparesis, paralysis of the tail, or turning and twisting movements. Most of the unimmunized mice died within 28 days after implantation; only a few survived longer, and none lived to day 56 (Fig. 2).

Both clinical and histologic criteria were used in appraising the effect of immunization. Clinically, growth of tumor was graded as "inhibited" when time of survival was longer than 28 days, and as "tumor rejected" when the mouse survived longer than 56 days after implantation. In the histologic study, the tumor was recorded as present only when there was histologic evidence of its presence. Deaths within a few days after implantation of tumor were considered to be caused by the surgical procedure and were

* Bacto adjuvant, complete (Difco).