A SIMPLE METHOD OF CONTINUOUS PASSAGE OF A HUMAN TUMOR THROUGH IMMUNOCOMPETENT MICE

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The creation of simple and inexpensive methods of growing human tumors in animals has for a long time been one of the aims of cancer research. Rejection of a xenograft of many human tumors can be overcome by the use of nude mice. However, the special equipment needed and the creation of conditions for keeping such mice often make their use difficult. Thymectomy on mice with additional procedures also can create the conditions for growth of a human tumor, but this approach is laborious and not always reproducible. The development of simpler and more reliable methods of overcoming the immunologic barrier to xenografting is essential in order to facilitate experimental work with human tumors.

The nonpigmented melanoma BRO, obtained from a primary focus in an untreated patient, grows quickly in nude mice [7] and in immunocompetent mice subjected to immunodepression in various ways [2, 3, 8] or to the development of tolerance [1].

In this paper we describe a simple method of maintaining growth of a human BRO melanoma implanted beneath the renal capsule (RC) of immunodepressed (ID) mice. By the use of this method, a human tumor can undergo continuous passage and be used for various kinds of research.

EXPERIMENTAL METHOD

(CBA × C57BL/6)F_1 hybrid mice aged 2-4 months and C57BL/6 mice were used. Cells of melanoma BRO [7] were obtained from nude mice or by culture in medium RPMI with 10% fetal serum, followed by mounting in a fibrin clot [3]. Tissue of a KSML-O mouse mammary gland carcinosarcoma [4] was generously provided by the metastasization group of the All-Union Oncologic Scientific Center, Academy of Medical Sciences.

The animals received whole-body irradiation on a ^{137}Cs apparatus (dose rate 0.087 Gy/sec) 24 h before transplantation of the tumors beneath RC. When Cytosar ("Upjohn") was used, the preparation was given 2 days before irradiation [9].

The operation to implant the tumor beneath RC was performed as described previously [3] and its two mutually perpendicular diameters were measured on the day of transplantation and again 7-14 days after the operation, when the mice were sacrificed. During serial passage of the tumor, the necrosis which was sometimes found inside large tumors was removed, and the viable part of the tumor cut into pieces measuring about 1 mm^3 for further implantation beneath RC. In each group 3-6 mice were used. The results were subjected to statistical analysis by the U test.

Standard methods of cytological study of the material were used.

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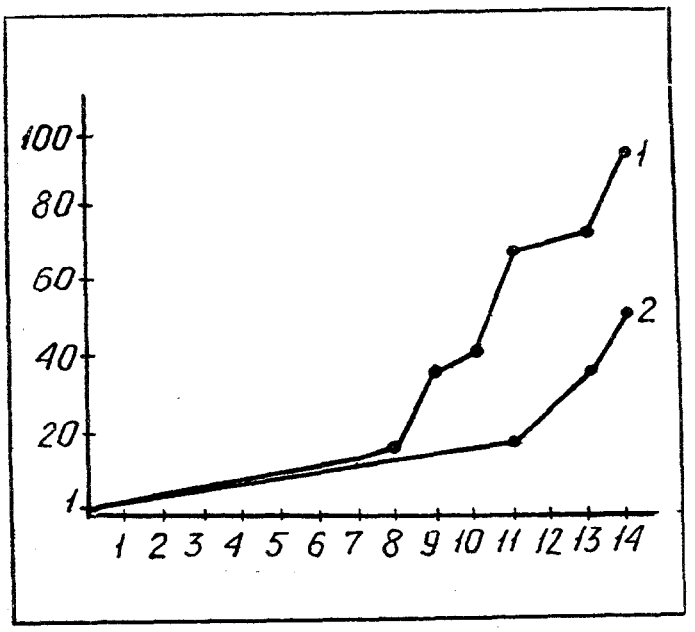

Fig. 1. Growth of BRO tumor beneath RC in mice previously receiving injection of Cytosar (200 mg/kg) and irradiation (8 Gy) (1) and in mice receiving irradiation only (7 Gy) (2). Abscissa, days after implantation of tumor. ordinate. relative volume of tumor.

EXPERIMENTAL RESULTS

In preliminary experiments on (CBA × C57BL/6)F_1 mice receiving an injection of Cytosar (200 mg/kg body weight) and irradiated (8 Gy), growth of an allogeneic mouse KSML-O tumor was observed after implantation beneath RC on 11 successive occasions. On the 13th day after transplantation the mean volume of the tumor (10 mice altogether) was 17 times greater than the initial dimensions.

Under the same conditions of immunodepression (ID) a melanoma BRO xenograft underwent 25 successive passages: the time of growth of the tumor in vivo lengthened from 7 to 14 days. Growth of BRO was faster than that of the KSML-O tumor (Fig. 1), and continued until the 14th day after transplantation, when the mean volume of the tumor was increased by 94 times compared with its original size. In all 127 mice in which growth of the transplant continued for 13-14 days the tumor was greatly enlarged. The average loss of mass 7 days after transplantation (8 days after irradiation) in ID produced in this way was 14%, and the original mass was completely restored 14 days after transplantation (15 days after irradiation).

In mice subjected to irradiation only (7 Gy) the BRO xenograft underwent 23 successive passages. Growth of the melanoma was much less ($p < 0.05$) than in mice receiving Cytosar and irradiation (8 Gy). The mean volume of the tumor 14 days after transplantation was 48 times greater than initially (Fig. 1). On the 14th day after transplantation there was no significant loss of mass compared with the original mass.

The BRO melanoma underwent eight consecutive passages also in C57BL/6 mice, which are less resistant to the tumor than (CBA × C57BL/6)F_1 hybrids. If the mice were given Cytosar and irradiated (8 Gy) 14 days after transplantation the mean volume of the tumor increased by 204 times compared with the initial size, and the mean loss of mass 14 days after transplantation was 7%. With Cytosar in a dose of 200 mg/kg and irradiation in a dose of 8 Gy, 6.3% of the C57BL/6 mice died compared with a mortality of 1.8% among (CBA × C57BL/6)F_1 hybrids.

Cytologic investigation of the various BRO grafts revealed a large number of polymorphic anaplastic malignant cells with large nuclei and nucleoli. Mitoses were observed; no pigment was found. No macrophages likewise were found, but only solitary lymphocytes in a few preparations. On the whole the cytologic picture corresponded to the characteristics of the BRO melanoma described previously [3].

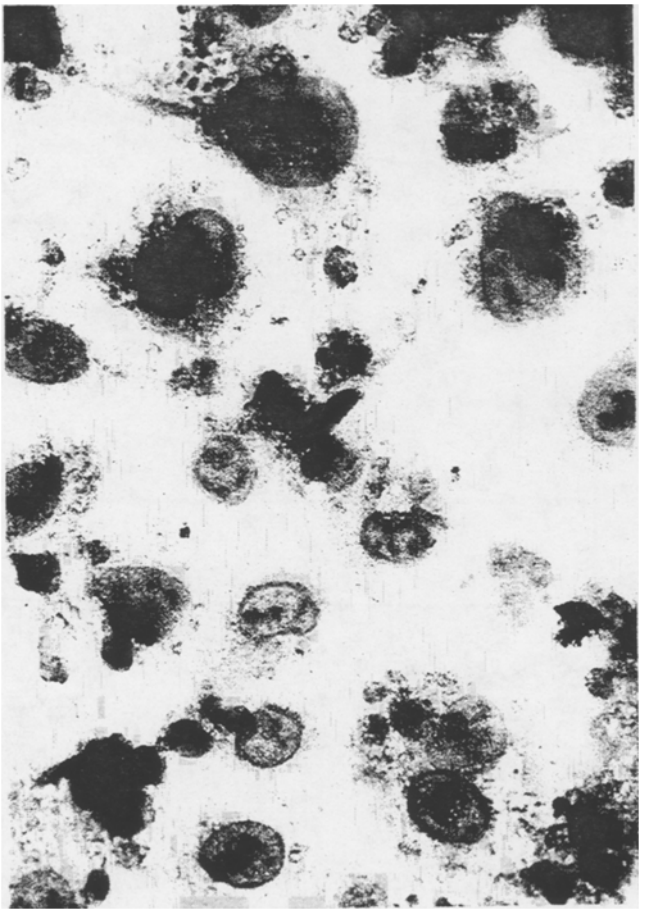

Fig. 2. BRO cells cultured after 11 successive passages beneath RC. 250×.

To test the viability of the BRO cells, a tumor obtained after the 11th passage was cut into pieces and small fragments were cultured. Cytologic investigation of cells in the culture revealed polymorphic malignant cells with nucleoli and gemmation (Fig. 2), similar to the description given above.

The results show that a human tumor can be grown continuously beneath RC in immunocompetent mice subjected to a simple type of ID.

In the case of whole-body irradiation with nonlethal doses, ID is incomplete and temporary [5]. With subcutaneous transplantation of the human tumor into irradiated mice, virtually complete rejection of xenografts was found as early as on the 10th day [10]. After transplantation every 8 days, six of 100 different human tumors were successfully transplanted subcutaneously four consecutive times, and one tumor as many as six times. A combination of injection of cortisone with irradiation of the rats created conditions of ID enabling growth of 90% of human tumors when transplanted subcutaneously. Growth of the tumor continued for 12-30 days, and one adenocarcinoma underwent four successful passages [11].

It was shown previously [2] that a BRO melanoma grew rapidly in irradiated immunocompetent mice after injection of a relatively small quantity of tumor tissue, about 250 mg per mouse. At autopsy massive carcinomatosis was found in the peritoneal cavity, leading to an agonal state and death of some animals. Neither lymphocytic infiltration nor other signs of immunologic rejection were found throughout the period of observation until the 19th day after transplantation.

In the present experiments transplantation of the tumor beneath RC was chosen, as a method providing favorable conditions for rapid adaptation of the tumor to its new location. Because of the relatively small size of the transplant (about 1 mm^3) it is possible for essential nutrients to penetrate into the tumor, and thus to enable its growth without having to await the end of the latent period for induction and penetration of blood vessels into the

tumor. With the development of a new local blood supply beneath RC an abundant vascular network is available for providing the tumor with a constant supply of nutrients [6].

Injection of Cytosar 2 days before irradiation enables a large dose of irradiation to be used [9], and under these conditions of ID, growth of the BRO melanoma takes place more rapidly. The tumor grew faster still when implanted into C57BL/6 mice, which are less resistant to irradiation. These results suggest that the degree of growth of the BRO melanoma depends on the degree of immunodepression.

The absence of any marked signs of rejection during the period of observation, up to and including the 14th day after transplantation, supports the view that BRO cells possess an intrinsic immunodepressive effect [2].

The results described above show that a human tumor can undergo continuous and reproducible passage in irradiated immunocompetent mice. The use of a simple method of immunodepression and of the known method of tumor implantation beneath the renal capsule make it possible to grow certain rapidly proliferating human tumors without the use of nude mice or of more complicated conditions of immunodepression.

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IMMUNOMODULATING ACTIVITY OF EXOGENOUS CERULOPLASMIN IN MICE WITH EXPERIMENTAL TUMORS

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The development of immunodepression is an essential manifestation of the systemic action of a tumor on the host. Restoration of disturbed activity of the immune system with the aid of various immunomodulators is an important step in the treatment of tumors. Data on the immunopotentiating and antitumor action of ceruloplasmin (CP), a blood plasma protein which performs several important biochemical functions in the body, are of great interest [2, 7-9, 11]. However, information on the immunomodulating properties of CP during growth of tumors is

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