EFFECT OF ANTI-HOST REACTION OF THE GRAFT ON TRANSPLANTATION IMMUNITY TO HETEROLOGUS TUMORS

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UDC 612.6.02.017.1:616-006-092.9

Although the problem of grafting heterologous tumors has been extensively investigated, little progress has been made in this field [1, 2]. The best results have been obtained when the recipients were given cortisone or a combination of cortisone with irradiation [1-3, 13, 14], and also in the production of acquired immunologic tolerance in newborn animals and embryos [4, 5, 9, 10]. However, all the methods used to suppress transplantation immunity to tumor heterografts in mature organisms have yielded inconstant results. The search for new methods of suppressing the natural resistance of animals to tumor heterografts must therefore be undertaken as a matter of urgency.

It was previously shown that the reaction of the graft against the host developing after injection of parental spleen cells into F_1 hybrids depresses the ability of the hybrids to form antibodies and, to a lesser extent, depresses homotransplantation immunity, particularly in sublethally irradiated hybrids [6, 7-10].

In the present investigation an attempt was made to utilize the anti-host reaction of the graft for depressing transplantation immunity of mice to rat sarcoma M-1.

EXPERIMENTAL METHOD

The recipients used in the experiments were (C57BL/6 × CBA)F_1 hybrids and the donors of the parental spleen cells were CBA (H-2k) C57BL/6(H-2b) mice. The two parental lines of animals differed with respect to the strong (H-2) locus of tissue compatibility. The donor and recipient mice were of the same sex. The F_1 hybrids and their parent lines were obtained from the pure-line animal nursery (Stolbovaya station) of the Academy of Medical Sciences of the USSR.

In some experiments, to intensify the reaction of the graft, the mice of the parent CBA strain were first immunized with spleen cells of C57BL/6 mice (abbreviated to CBA-anti C57BL/6 donors were immunized with CBA spleen cells (C57BL/6-anti CBA). Usually three intraperitoneal injections were given, each consisting of 30×10^6 spleen cells.

The tumor used for implantation was a transplantable strain of rat sarcoma M-1 obtained from a tumor induced by 3, 4-benzpyrene [6]. The tumor strain was obtained from the tumor strains laboratory of the Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR. The tumor was transplanted into noninbred albino rats.

The hybrids were irradiated with a sublethal dose (500-600 R) of x-rays, and 24 h later they received an intraperitoneal injection of spleen cells of the parent strains. Preparation of the spleen cell suspensions was described previously [9].

The conditions of irradiation were: RUM-11 apparatus, voltage 180 kV, current 15 mA, filters 0.5 mm Cu and 1.0 mm Al, focus distance 40 cm, field 20 × 20 cm, dose rate 44-48 R/min.

At various times after irradiation and injection of the parental spleen cells the hybrids were inoculated (usually subcutaneously) with a suspension of tumor tissue in physiological saline or Hanks's solution (0.4 ml of 1:5 suspension each).

To obtain histological specimens pieces of tumor were fixed in 10% formalin and embedded in celloidin. Sections were stained with hematoxylin-eosin or by Van Gieson's method.

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vo. 64, No. 8, pp. 83-85, August, 1967. Original article submitted March 5, 1966.

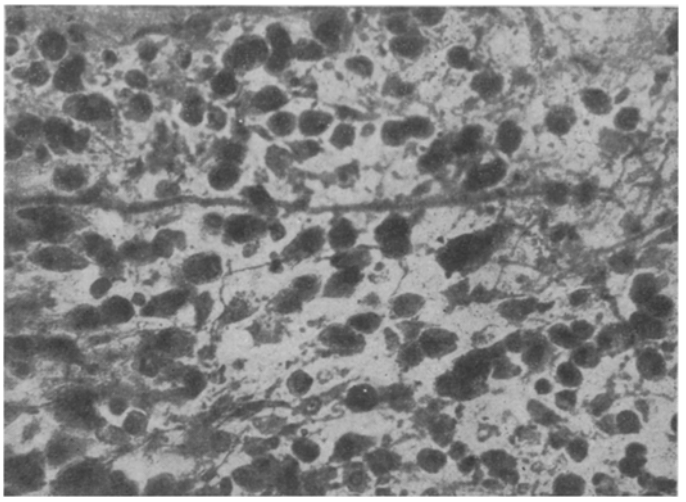

Fig. 1. Section through tumor at the site of inoculation of sarcoma M-1 into (C57BL/6 × CBA) F_1 hybrid after irradiation in a dose of 500 R and transplantation of parental spleen cells.

EXPERIMENTAL RESULTS

In the experiments (4) of series I the sarcoma M-1 was implanted immediately after transplantation of 20×10^6 parental spleen cells. In experiment N. 1 60 mice were irradiated with a dose of 500 R and 20 unirradiated hybrides served as controls. The donors of the parental strains were preliminarily sensitized by injection of spleen cells of the second parent strain. C57BL/6-anti CBA cells were injected into 20 hybrids, while CBA-anti C57BL/6 cells and a mixture of equal doses of the two types of cells were injected into 10 hybrids each. The controls included unirradiated normal hybrids, irradiated hybrids not receiving parental cells, unirradiated hybrids receiving C57BL/6-anti CBA cells, and irradiated hybrids receiving cells of isologous donors (10 mice in each group). The recipients were 2.5 months old. In this particular experiment all the irradiated recipients receiving injections of sensitized parent cells developed homologous disease, causing death of 36 of the 40 mice. The hybrids of the remaining groups survived. At the site of inoculation either no tumor was found or small nodules of infilatration could be felt, disappearing after 2-3 weeks. Only one mouse developed a tumor the size of a cherry, and this also was absorbed. However, in this experiment large conglomerates were found in the abdomen of 13 of the 36 hybrids which died, and these attained a weight of 350 g.

Histological examination showed that these masses consisted of the greatly enlarged pancreas, containing tumor-like nodules in which the cells differed sharply in their morphology from the original tumor cells. No infiltrative growth was found. Necrotic changes were seen at the periphery of these nodes, together with focal and diffuse hemorrhages.

In the other three experiments of this series the recipients were 5-6 months old and the dose of spleen cells was $36\text{-}75 \times 10^6$ (from unsensitized C57BL/6 donors, and in experiment No. 3 from CBA-anti C57BL/6 mice also). In experiment No. 2 progressive growth of the tumor was noted in 40%, in experiment No. 3 in 60%, and in experiment No. 4 in 90% of recipients. Histological examination revealed a typical polymorphocellular sarcoma, with areas of spindle-cell type (see figure). The tumors varied from the size of a cherry to that of a plum.

In experiments Nos. 2 and 3 the recipients developed a severe form of homologous disease from which they died. In experiment No. 4 a moderately severe form of homologous disease was observed, and during the second month after inoculation gradual absorption of the tumors took place.

In experiment No. 3, among the hybrids receiving intraperitoneal injections of tumor tissue after preliminary subcutaneous injection of similar doses, no tumor growth was observed in any animal. The impression was gained that intraperitoneal implantation of the tumor prevented the tumor cells from surviving at the site of subcutaneous inoculation.

In the experiments of series II, the sarcoma M-1 was inoculated into hybrids 37 days after sublethal irradiation and injection of a mixture of equal doses of CBA-anti C57BL/6 and C57BL/6-anti CBA cells (total dose 44×10^6). Progressive tumor growth followed by death of the animal was observed in 2 of the 9 mice, whereas after simultaneous inoculation of 10 control irradiated (500 R) hybrids and of 10 unirradiated hydrids not receiving spleen cells with the tumor, no tumor growth was seen in any of the animals.

Negative results were obtained in the experiments of series III in which the tumor was implanted into hybrids protected with isologous bone marrow after lethal x-ray irradiation, 58 (20 mice) and 42 (54 mice) days after irradiation and transplantation.

The results thus demonstrated that the anti-host reaction of the graft in sublethally inrradiated hybrids may be used with success for tumor heterografting, especially in conditions when inoculation of the tumor cells is carried out soon after irradiation and injection of parental spleen cells. An important finding is that successful results may also be obtained on hybrids 5-6 months old. However, if the hybrids survived the homologous disease, the tumors usually disappeared. In the present experiments irradiation did not facilitate the progressive growth of the tumor, even when lethal doses of radiation were given and this was followed by protection with isologous bone marrow.

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