TRANSPLANTATION OF THE BROWN-PEARCE RABBIT CARCINOMA TO RATS

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Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 52, No. 9,
pp. 94-97, September, 1961
Original article submitted July 15, 1960

The problem of heterotransplantation is one of the most important in experimental oncology. Although works in this area were begun a long time ago and are being intensely continued, lasting transferable heterogenic tumors, particularly human tumors to animals, do not exist. Several means of producing heterogenic tumors have been noted. Almost all of these are based on suppression of the transplantation immunity by the use of cortisone and roentgen rays [5, 7, 10, 13, 14, 16, 17].

Lately, "immunological tolerance" is used for the prevention of tissue incompatibility reactions associated with homo- and heterotransplantation [2, 4, 6, 11]. Further experiments in this direction, elucidating individual phases of heterotransplantation, are of major importance in clinical and experimental oncology.

Taking into consideration the immunological factors, in particular the necessity for suppression of the immune powers of the recipient in order for the heterotransplant to survive, we developed a method for transplanting the Brown-Pearce rabbit tumor to rats.

METHOD AND RESULTS

The reactions to sensitization and desensitization were selected as the basis of the experiments [1]. Four to six week old rats were subcutaneously injected with 1 ml of antigen, prepared from the rabbit tumor. As a rule, we determined the antigen's content of protein, which usually ranged from 11 to 15 mg per ml of antigen. Within 7-10 days after the first injection of antigen the rats received the same dose, administered into the caudal vein or into the heart. At this time we observed a minimal reaction, in the form of a temperature drop and sluggishness; in certain cases, 1-2 of the 10-15 experimental rats died. Within 2 hours after the repeat injection of antigen we tested the completeness of the desensitization, and after another two hours the rats were inoculated subcutaneously with the Brown-Pearce rabbit carcinoma, following the generally accepted method.

In order to clarify what effect the interval of the repeat antigen injection (desensitization) has on the ability of the tumor to be transplanted, it was injected after 3, 7, 10, 15, 20, 27 and 30 days. The best results of transplantation were obtained by injecting the antigen 7-10 days after the initial administration. When the desensitization was performed 3, 15, 20, 27 or 30 days afterward, with subsequent transplantation of the heterogenic tumor, we observed only minimal growth of the tumor, and complete resorption by the tenth day after the inoculation. M. A. Frolova and co-authors have also demonstrated [9] that the peak of an organism's allergic response occurs within 7-14 days after the first injection of antigen.

Thus, the first injection of antigen (sensitization) causes a reaction to the foreign proteins on the part of the immune powers of the recipient; the second injection of antigen on the 7-10th day (at the height of the sensitization) suppresses the immune powers of the recipient to the point where it is possible for the heterogenically transplanted tumor to develop.

We not only used tumor material as the antigen, but also rabbit serum from animals with the transplanted Brown-Pearce carcinoma, and, for the control, serum from healthy rabbits and horse serum. We performed the ex-

periments on 600 rats. The goal of our investigations was, first, to employ the described method and obtain the Brown-Pearce tumor in rats for a series of generations, making it possible to study their biological and biochemical properties; secondly, to test the feasibility of transplanting the heterogenic tumor from the prepared rats to healthy ones.

Above all it must be noted that in using this method we obtained successful transplantations only if the tumor destined for transplantation was taken from a rabbit that had had the tumor for a long period of time and in whom there were widespread metastases in the internal organs. This observation was confirmed by numerous experiments which we carried out, as well as by certain data in the literature. Thus, Green [11] connected the development of the capacity for heterotransplantation with the initiation of metastasis, and even proposed that successful transplantations be considered as a prognostic test. Other authors [3, 7, 8] have felt that in order to have successful transplantations a number of factors are necessary: selection of the experimental animal, the production of a lowered resistance, and the site of injection of the transplant, are those to which V. N. Stepina, for example, attaches basic importance [7, 8]. While in agreement that the production of a lowered resistance is of essential importance, we cannot avoid mentioning the necessity of using the tumor of a rabbit having widespread metastases for the transplantation.

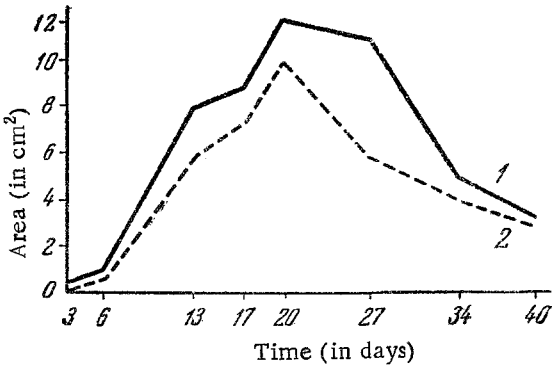

Fig. 1. Development of the Brown-Pearce rabbit tumor in the prepared rats. 1) Rat No. 1; 2) rat No. 2.

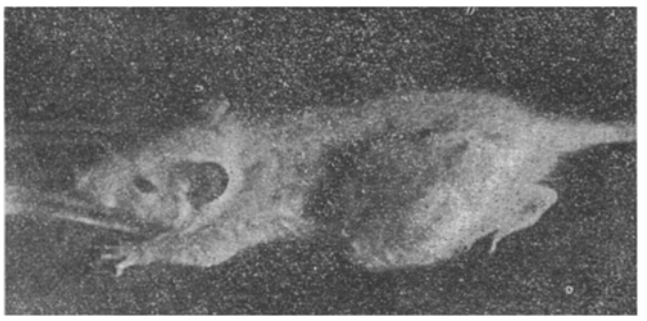

Fig. 2. Brown-Pearce rabbit tumor developing in a prepared rat. Third generation.

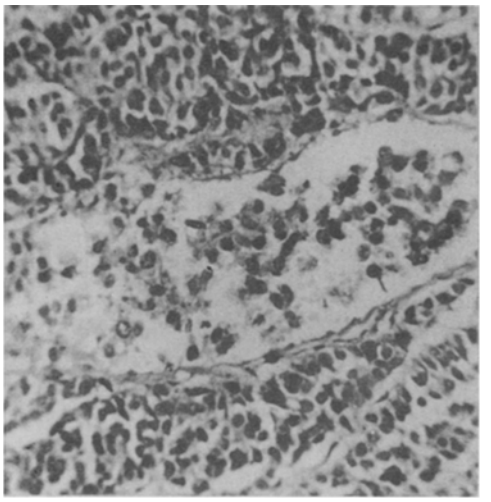

Fig. 3. Histological structure of the Brown-Pearce rabbit tumor developing in a prepared rat. Tumor thrombus. Magnification 85 × 8.

When the rats were prepared by the method indicated above, palpable tumors appeared in 80% of the animals on the 4-5th day after the transplantation. The dimensions of the tumor increased, attaining their greatest levels by the 16th to 20th day.

Fig. 1 shows the development and regression of the tumors in two rats. The growth of the tumor occurred much more energetically and in a shorter period of time (16-20 days) than the regression, which lasted 1½ to 2 months. The weight of the tumor at the time of its maximal development reached 10-20 grams. A short time after the tumor became palpable its growth became very slow, giving the impression that the transplantation had failed (latent period); then an acceleration in its growth occurred, lasting up until the 16-20th day.

Having proven, in a large number of experiments (approximately 200 rats), the feasibility of obtaining the Brown-Pearce tumor in rats by the method we developed, we attempted to transplant the heterogenic tumors through a series of generations.

Twenty rats, four to six weeks of age, were placed in each group. After preliminary preparation, the tumor was transplanted from the rabbit to the rats, and thence from rats to rats. On the 7-8th day after transplantation of the Brown-Pearce tumor to the prepared animals we observed well developing tumors in 80% of the rats, attaining weights of 2-3 grams. For the next transplantation (2nd generation) we sacrificed a rat with a succulent, viable tumor. The "take" rate of the transplants in the 2nd generation was equal to 70%, in the 3rd generation - 70%, in the 4th - 60 - 70%. The survival rate of the transplants was followed through 8 generations.

Fig. 2 shows the heterogenic tumor formed in a prepared rat (3rd generation) on the 20th day of development. The dimensions of the tumor were 4.5 × 2.7 cm, and it weighed 19.7 grams. The tumor developing in prepared animals was more viable. In histological preparations we often encountered mitoses and blood vessels of various calibres. In many places the tumor grew through the walls of the vessels. In other vessels we observed tumor thrombi (Fig. 3). In some places the tumor grew through the capsule, where there were also a large number of vessels of varying calibre.

Transplantation of the tumor to unprepared rats was performed in this manner: first it was transplanted to prepared rats several times, and then from them to the healthy rats. In this case the percent of "takes" was lower than in the prepared animals; in the 1st and 2nd generations (prepared rats) it was 80%, in the 3rd generation (unprepared) – 60%, in the 4th – 50%. The duration of tumor development in the unprepared animals was shortened to 10 - 12 days, instead of the 16 - 20 days seen in the prepared rats.

In histological investigations of preparations made from a tumor developing in an unprepared rat we observed both large and small tumor foci; a portion of the tumor cells were in a dystrophic state, and necrosis was sometimes observed. Mitoses were singular. The number of tumor cells sharply decreased from the center to the periphery.

Thus, in prepared rats the heterogenic tumor develops better and is more viable. These properties are gradually lost with successive passages to unprepared rats.

The Brown-Pearce tumor, developing in rats through a series of generations, retains its initial properties when transplanted to rabbits. This was proven by systematic subcutaneous transplantation of the heterogenic tumors from various generations back to rabbits, and by study of the histological structure of these tumors. Using these transplants, the rabbit subjects usually died from metastases to the internal organs. Survival of the transplants occurred in 80% of the cases.

As was indicated above, we used not only tumor material for the antigen, but also serum from rabbits with the transplanted Brown-Pearce carcinoma. In these experiments the survival rate of the Brown-Pearce tumor transplants to rats was equal to 60%. As a control we transplanted the Brown-Pearce tumor to healthy rats and to rats which had previously received only one injection of the antigen subcutaneously. In these cases we observed an insignificant thickening, which was resorbed by the 5th day after the inoculation, or complete absence of tumor development. When the antigen used was the serum from healthy rabbits or horse serum, we failed to observe any growth of the heterogenic tumors. This again confirmed the specificity of the organism's reaction to the tumor antigen.

Thus, the method which we have developed makes it possible to obtain the Brown-Pearce rabbit tumor in rats. The tumor attains large dimensions, and is resorbed slowly; this makes it possible to study the dynamics of changes in its biological and biochemical properties.

SUMMARY

One of the most important problems in experimental oncology is tumor heterotransplantation. The authors developed a method for transplanting rabbit Brown-Pearce tumors to rats. Heterogenic tumors were thus obtained in rats. By the 16th - 29th day after inoculation their weight was from 10 to 20 gm. Later on a slow resolution of the tumor occurred, lasting for 1.5-2 months and more; this enabled them to study dynamic changes of its biological and biochemical properties. The tumor was maintained in a number of generations of previously prepared rats, as well as by its transplantations from the prepared rats to the healthy ones. The tumor taken in the prepared rats constituted 70%. When transplanted to rabbits Brown-Pearce tumor developed in generations of rats retains its primary properties.

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