A HISTOLOGICAL STUDY OF THE BROWN-PEARCE TUMOR OF RABBITS DEVELOPING IN RATS

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No general agreement has yet been reached on the questions of the morphological changes in transplanted tumors and the importance of the local connective-tissue and vascular reactions of the recipient in the process of the survival or death of heterografts. Some writers [4, 9] consider that one cause of the death of a heterogenous tumor transplant in a recipient is the lack of a blood supply. Merwin and Hill [17], on the other hand, point out that a good blood supply favors the absorption of the graft as a result of the penetration of antigens from the graft into the blood stream, with the subsequent development of reactions of immunity.

R. M. Radzikhovskaya [8], citing various foreign sources, stresses the great importance of lymphocyte infiltration in the absorption of the tumor. Medawar [15], however, is doubtful about the importance of lymphocytes as a protective factor.

Simonsen [20] notes that during a lymphocyte reaction there is also a connective-tissue reaction in the form of inflammation around the graft. Yu. M. Vasil'ev and L. V. Ol'shevskaya [5, 6] who studied the latter phenomenon, found that the initial inflammation facilitated growth of the tumor. These workers attach great importance to the lymphocytes, considering that they play a direct part in causing death of the graft. Yu. M. Vasil'ev [7] points out that the lymphoid tissue of the recipient plays a decisive role in the development of specific immunity, which is largely responsible for the death of the heterogenous graft, and that the most characteristic of the local connective-tissue reactions is lymphoid infiltration.

A. I. Ageenko [3] studied the pattern of the protective reaction of the reticulo-endothelial system of the recipient after transplantation of tumors and concluded that cortisone causes marked inhibition of the cell reactions of the connective tissue around the graft and thereby contributes towards its survival.

In the present investigation we studied the histological changes in a Brown-Pearce tumor of rabbits when growing in rats, paying attention to the morphological changes in the heterogenous graft, to the connective-tissue reactions around the implanted tumor, the development of the stroma and the vascularization.

EXPERIMENTAL METHOD

The possibility of producing a heterogenous Brown-Pearce tumor of rabbits was demonstrated in our previous reports [1, 2].

Rats aged 3-4 weeks were given an intramuscular injection of antigen prepared from a rabbit tumor (Brown-Pearce strain). Usually 1 ml of antigen contained from 11 to 15 mg of protein. From 7 to 10 days after the first injection of antigen, at the height of sensitization, the same dose was injected into the caudal vein or the heart. Two hours after the second injection of antigen and tests for completeness of desensitization, a Brown-Pearce tumor of rabbits was implanted into the rats subcutaneously. During the preparation of the rats by this method, on the 4th-5th day after inoculation, palpable tumors developed in 80% of the animals. Consequently, the reinjection of antigen at the height of sensitization suppressed the immune powers of the recipient to such an extent that it enabled a heterogenous tumor to develop. The Brown-Pearce tumor reached its largest size (10-12 g) in the rats on the 16th-20th day after inoculation. Growth of the tumor took place far more energetically than regression, which

continued for 1.5-2 months. This allowed us to study the histological changes taking place in the tumor for a long period (from the time of inoculation of the tumor to its total death), and also during inoculation into a series of generations.

For histological investigation of the tumors, the rats were sacrificed on the 3rd, 7th, 10th, 15th, 20th, 30th, 40th, 50th, and 65th days after inoculation. Pieces of the tumor were fixed in a 10% formalin solution. Preparations were stained with hematoxylin-eosin, by Van Gieson's method, and for fat with Sudan III.

EXPERIMENTAL RESULTS

The basic transplantable Brown-Pearce tumor consisted mainly of round, hyperchromic carcinoma cells. The stroma was poorly developed. Here and there a lymphocytic infiltration was present. Necrobiosis and necrosis were most marked at the periphery. In some areas tumor cells infiltrated the capsule surrounding the tumor.

The histological study of sections prepared from various parts of the heterogenous tumor at different times after inoculation showed that it mainly preserved its primary structure.

On the 2nd-3rd day after inoculation of the tumor growth of the tumor tissue began and the first sign of vascularization appeared. A marked inflammatory reaction was present around the heterogenous graft.

On the 7th day the tumor consisted of juicy, round, well-stained cells. Mitoses were frequent and many blood vessels of different caliber were present, sometimes containing malignant thrombi. The stroma was reticular and almost invisible. Lymphocytes, fibroblasts and histiocytes were scattered between the vessels. Small necrotic foci were seen only at the periphery of the tumor (see figure, a).

On the 10th day further intensive development and growth of the heterograft was observed macroscopically. Under the microscope the tumor consisted of well-stained round cells with readily distinguishable nucleoli and frequent mitoses. The stroma of the tumor in places consisted of a delicate reticulum of connective tissue and many blood vessels of different caliber. Some of these contained vast numbers of tumor cells. Few lymphocytes, histiocytes and fibroblasts were seen. In other areas there were bands of connective tissue containing blood vessels in which the blood was replaced by tumor cells. In these places many leukocytes, lymphocytes and cell fragments were seen.

On the 15th day, macroscopically the growth of the tumor was slower, although its size continued to increase and its weight reached 10-12 g. Microscopically, large groups of juicy, well-stained tumor cells, rich in mitoses, were seen. The stroma was well developed in places. Bands of connective tissue were observed, rich in lymphocytes, fibroblasts, and histiocytes, with solitary blood vessels. In the center of these bands a group of modified tumor cells was observed. Groups of dystrophic tumor cells with signs of necrobiosis and necrosis were sometimes observed at the periphery, together with varying numbers of leukocytes and cell fragments (see figure, b, c).

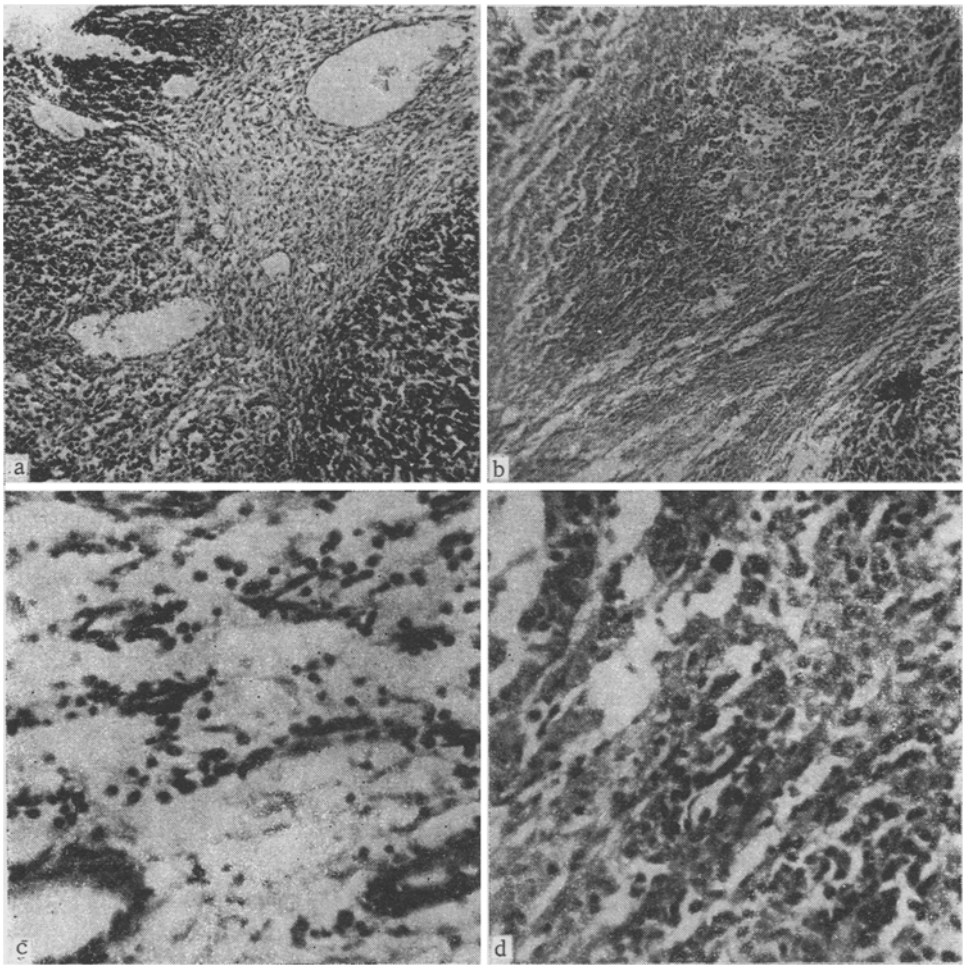
On the 20th day, macroscopically the growth of the tumor had ceased, and gradual and prolonged regression begun, which continued for 1-2 months. Microscopically, the number of living, well-preserved tumor cells showed a decrease. Dystrophic cells were predominant, frequently showing signs of necrobiosis and necrosis. In the foci of destruction, and also at the periphery of the tumor, many small and medium-sized lymphocytes, a few leukocytes and cell fragments, and an occasional erythrocyte were seen. Groups of tumor cells were preserved in the connective tissue, where the number of cells had increased sharply. Hence, on the 20th day after inoculation of the tumor there was a marked intensification of the dystrophic changes in the tumor cells, although many of them remained juicy and viable.

On the 30th-50th days, regression of the tumor cells gradually increased, although at these times viable tumor cells with the presence of mitoses could still be seen (see figure, d). Large numbers of blood vessels of different caliber were present, often containing normal and abnormal tumor cells within their lumen.

On the 65th day the tumor cells were in a state of necrobiosis and necrosis. Around the vessels were many leukocytes, histiocytes and, in particular, lymphocytes.

Hence the study of histological preparations of a Brown-Pearce tumor at various periods after inoculation into rats showed that degradation of the tumor cells began at the 15th-20th day. By the 40th-50th day, most of the transplanted tumors had become absorbed. The remaining tumors were soft in consistency and consisted histologically largely of necrotic cells, extensive infiltration of leukocytes and lymphocytes, and cell fragments.

In the experiments of Yu. M. Vasil'ev [7], the connective-tissue reactions in rats after inoculation of a Crocker's sarcoma and PCM mouse carcinoma were studied at intervals. This worker divided the reactions of the organism to the heterograft into three phases: aseptic inflammation (until the 4th day); lymphoid infiltration (4th-6th day); and the development of granulation tissue around the dying graft (9th-20th day).



A Brown-Pearce tumor of rabbits in a rat on the 7th day after inoculation (a). Tumor tissue is seen at the periphery, and at the center is a proliferation of fibrous tissue rich in vessels. Some vessels contain tumor cells. 360×. The same tumor on the 15th day after inoculation (b), with proliferation of fibrous tissue, rich in lymphocytes, between the tumor foci. 360×. The same preparation under high power (c). Many lymphocytes, mainly middle-sized. 560×. A Brown-Pearce tumor in a rat on the 30th day after inoculation (d). Loose connective tissue rich in small lymphocytes, histiocytes, and fibroblasts. Solitary abnormal tumor cells. 560×.

The study of our material showed that the first phase of the local connective-tissue reaction, expressed as aseptic inflammation, commencing growth of connective tissue, and vascularization, took place uniformly in all cases, and at times coinciding with those given by Vasil'ev. The second wave of lymphocytic infiltration, however, of the greatest importance of the development of the heterogenous tumor, began much later (on the 10th-15th day), and from this time the growth of the tumor began to be slowed. As a rule, lymphocytic infiltration was observed in places where the tumor cells were sharply modified. From the 20th to the 65th day, groups of tumor cells were preserved only in the connective tissue, in which the number of cells was considerably increased. The most marked dystrophic changes were present at the margin of the tumor nodule, where the lymphocytes were most numerous.

It is possible that the histological changes which we observed in our specimens were to some extent due to the method which we used to obtain heterogenous tumors. For instance, lymphoid infiltration began much later, and regression of the heterogenous tumor also began at these periods. Consequently, our findings confirm the opinion of many researchers [7, 18, 6] that death of the heterogenous graft is associated with increasing lymphoid infiltration.

We observed vascularization in nearly every tumor we studied, and in most cases the vessels were thrombosed with tumor cells. In the clearly stages of development of the grafted tumor (until the 20th-30th day), the thrombi consisted mainly of viable tumor cells. In our material, however, we found that vascularization had no significant role in the regression of the heterograft.

SUMMARY

A dynamic study of histological preparations of a Brown-Pearce tumor, transplanted in rats, indicated that initial signs of tumor regression commence from the 15th day and complete death of tumor cells is noted by the 40th-65th post-transplantation day. In view of the abovesaid the transplantation of tumors by this method is possible in a number of generations even on the 10th-12th day after heterotransplantation. Notwithstanding good tumor vascularization and the presence of tumor thrombi in the vessels, necrosis and death of the cells occur by the 40-65th day. A prevalence of lymphoid infiltration is observed in the areas where the changes of the tumor cells are marked.

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