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HEMATOPOIETIC TISSUE REACTIONS ASSOCIATED WITH GROWTH OF SYNGENEIC TRANSPLANTABLE TUMORS IN MICE

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Growth of a hemangiopericytoma in syngeneic (CBA× C57BL/6j)F_1 male mice led to regular changes in hematopoiesis: an increase in the weight and number of the spleen cells, an increase in the number of colony-forming units (CFU) in the spleen, intensification of myelopoiesis in the spleen, and the development of leukocytosis with a sharp increase in the number of polymorphonuclear granulocytes in the circulating blood, i.e., a "leukemoid reaction" syndrome. This syndrome developed also when tumor cells were injected into a splenectomized host. A leukemoid reaction, although less marked, also was found in the late stages of development of a hepatoma, transplanted into syngeneic (CBA× C57BL/6j)F_1 male mice. Meanwhile, transplantation of a syngeneic strain of carcinoma of the bladder into the same mice did not lead to the development of a leukemoid reaction.

KEY WORDS: hemangiopericytoma; hepatoma; carcinoma of the bladder; leukemoid reaction.

During growth of certain solid tumors in animals and man changes in hematopoiesis – the so-called leukemoid reaction – have frequently been described [4, 5, 10, 12]. Leukemoid reactions in mice were characterized by a high leukocytosis, with the dominance of polymorphonuclear neutrophils, splenomegaly, an increase in the number of stem cells in the spleen and bone marrow, and extramedullary hematopoiesis in the liver, kidneys, and lungs [1, 3, 7-9].

The object of the present investigation was to study the leukemoid reaction in mice with transplantable tumors of strains of hemangiopericytoma, hepatoma, and carcinoma of the bladder. The strains were obtained in the writers' laboratory and maintained by serial passage through syngeneic recipients [2].

EXPERIMENTAL METHOD

Experiments were carried out on adults (CBA× C57BL/6j) mice weighing 20-22 g reared at the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR. The mice were divided into two groups: the mice of group 1 were inoculated with tumors, the mice of group 2 (control) remained intact. Tumors were transplanted subcutaneously into male mice: hemangiopericytoma, $1 \cdot 10^6$ cells per animal; carcinoma of the bladder

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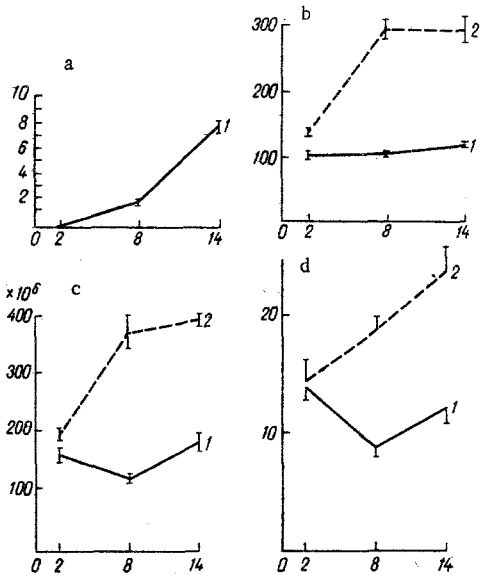

Fig. 1. Changes in spleen connected with growth of hemangiopericytoma (18th and 21st passages). Graphs drawn from combined results of two experiments: 1) control; 2) experiments. Abscissa, days after transplantation of tumor; ordinate: a) change in weight of tumor, b) weight of spleen, c) absolute number of nucleated cells in spleen, d) relative number of CFU per 10^6 nucleated spleen cells.

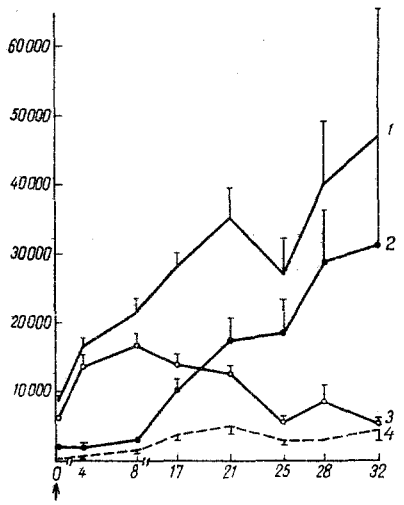

Fig. 2. Changes in blood leukocyte count connected with growth of hemangiopericytoma (9th passage): 1) leukocytes; 2) granulocytes; 3) lymphocytes; 4) monocytes. Abscissa, days after transplantation of tumor; ordinate, number of leukocytes in 1 mm^3 blood.

and hepatoma, 0.5 ml of a 50% homogenate of tumor tissue per animal. At various times after transplantation of the tumor cells the state of hematopoiesis in the mice was determined with respect to several indices. The number of leukocytes in 1 mm^3 peripheral blood was counted and the leukocyte formula calculated. On the basis of the results the absolute numbers of the various types of leukocytes in 1 mm^3 blood was calculated. Four mice from each group were sacrificed at various times after transplantation of the tumor cells. The spleen and femora were removed from the killed animals. The spleen was weighed, the absolute number of nucleated cells in the spleen was determined, and the qualitative composition of the cells in squash preparations of the spleen and films of the bone marrow was studied. Blood and bone marrow films and squash preparations of the spleen were stained with azure–eosin. A differential count was undertaken on 200 cells in blood films and 1000 cells in bone marrow films and squash preparations of the spleen. In the experiments with transplantation of the hemangiopericytoma and hepatoma the number of colony-forming units (CFU) in the spleen also was determined, by the method of cloning hematopoietic cells in spleens of lethally irradiated mice [11]. Females weighing 21-22 g were irradiated in a dose of 1200 R. An intravenous injection of 10^6 living nucleated spleen cells was given to the mice 24 h after irradiation. The suspension of spleen cells consisted of the combined pool of cells obtained from four mice of the same group at the same time of day. The suspensions were made up under aseptic conditions in the cold in medium No. 199. Living cells were counted in a Goryaev's chamber under water immersion. Colonies in the spleen were counted after 8 days. The numbers of endocolonies on average did not exceed 0.1 per spleen. The results were subjected to statistical analysis and the significance of differences was assessed at the $P < 0.05$ level by Student's method.

EXPERIMENTAL RESULTS

The main changes in the spleen and blood of the mice connected with growth of the hemangiopericytoma are illustrated in Figs. 1 and 2. During growth of the tumor in the mice the weight of the spleen and the number of cells in it increased considerably. The reaction of the spleen was so active that its weight and absolute number of cells were significantly higher than the control levels as early as 2 days after transplantation of the tumor. The relative and absolute numbers of CFU also increased (Fig. 1). The study of the cell composition of blood of the mice with tumors showed that as the tumor grew the leukocyte count increased, so that by death of the animals it averaged 44,000 cells/mm^3 blood, and in exceptional cases reached 110,500 cells/mm^3. The increase in the number of leukocytes was mainly connected with an increase in the number of polymorphs and monocytes. The number of mature forms of neutrophils was 10 times, and the number of monocytes 10-20 times greater than normal. The lymphocyte count at first increased, following the leukocytosis, but later it fell and was back to normal by the end of the experiment (Fig. 2).

Preliminary splenectomy (8 weeks before transplantation of the tumor) did not affect the development of the leukemoid reaction in the blood but inhibited growth of the tumor and prolonged the survival of the animals.

Squash preparations of the spleen of the mice with tumors showed increased proliferation of cells of the myeloid series, monocytes, and reticular cells. The predominant cell types were myeloblasts, promyelocytes, myelocytes, and polymorphs. The number of lymphocytes and megakaryocytes remained unchanged. An increase also was observed in the number of juvenile forms of the erythroid series (proerythroblasts, basophilic erythroblasts, polychromatophilic erythroblasts, and oxyphilic erythroblasts). In squash preparations of bone marrow, unlike those of the spleen, no increase in the intensity of myelopoiesis was found. The number of myeloblasts, promyeloblasts, and myelocytes was unchanged, whereas the number of stab cells and polymorphs fell below the control levels ($P < 0.01$). There was a marked increase in the number of blast cells and megakaryocytes in the mice with tumors. The study of the blood and spleen of mice with a hepatoma revealed in principle the same changes: an increase in weight of the spleen and in the absolute number of cells in it, an increase in the relative and absolute number of CFU. However, these changes differed somewhat from those observed during growth of the hemangiopericytoma. The increase in the number of CFU was observed only in the latest stages, when the tumors were very large and contained many necrotic areas and hemorrhages. A high leukocytosis was never observed in the blood (the initial level was 12,800 cells/mm^3, rising to 26,900 cells/mm^3 blood on the 43rd day). The leukocyte formula also showed a shift toward mature neutrophils. After transplantation of a carcinoma of the bladder, no increase in weight of the spleen or in the absolute number of cells in it was observed. The total number of leukocytes in the blood was not higher than normal, although the blood formula was "reversed" with an increase in the number of mature granulocytes. For instance, on the 50th day there were 60% of granulocytes compared with an initial 10-20%.

Growth of the transplantable syngeneic tumors studied in these experiments thus led to the development of a leukemoid reaction. Significant changes in hematopoiesis, moreover, were found in the spleen. The leu-

kemoid reaction syndrome in mice with hemangiopericytoma, in the writers' opinion, is a convenient experimental model with which to study and identify factors capable of influencing the number of stem cells and maturation of monocytes and granulocytes. Besides its purely theoretical interest, the isolation of such factors could be of practical interest, for they could be used to influence the regeneration of hematopoietic tissue when damaged in the course of cytostatic therapy. The writers have suggested that factors of humoral nature are responsible for the development of the leukemoid reaction in mice with tumors. Delmonte [6] succeeded in isolating from a transplantable mammary gland carcinoma a humoral factor capable of inducing proliferation of stem cells and discharge of leukocytes into circulation. However, the etiological role of other agents and, in particular, of viruses, which are frequent "passengers" of transplantable tumors, cannot be ruled out.

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IMMUNOLOGIC REACTIVITY AND ADRENOCORTICAL FUNCTION IN THE EARLY PERIOD OF CHEMICAL CARCINOGENESIS

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A parallel study was made of the level of immunologic reactivity and the state of adrenocortical function in BALB/c mice 7 days after a single injection of various doses of methylcholanthrene. Definite correlation was found between the degree of immunodepression induced by different doses of methylcholanthrene and determined as the number of antibody-forming cells in the spleen of the experimental mice and the 11-hydroxycorticosteroid concentration (both free and bound) in the blood plasma of the mice 7 days after injection of the carcinogen. These results may indicate that adrenocortical hormones play an important role in the pathogenesis of immunodepression induced by a chemical carcinogen.

KEY WORDS: carcinogenesis; immunodepression; antibody-forming cells; 11-hydroxycorticosteroids.

Immunodepression is an important pathogenetic stage in the formation and progression of the neoplastic process and it accompanies the development of the neoplasm from the moment of generation of the transformed cell until death of the host as a result of the formed tumor.

The mechanism of development of immunodepression due to the presence of a tumor differs from that of immunodepression associated with the induction of a tumor by chemical carcinogens or irradiation. In the

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